**Component-Based Syntheses of Trioxacarcins**

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by

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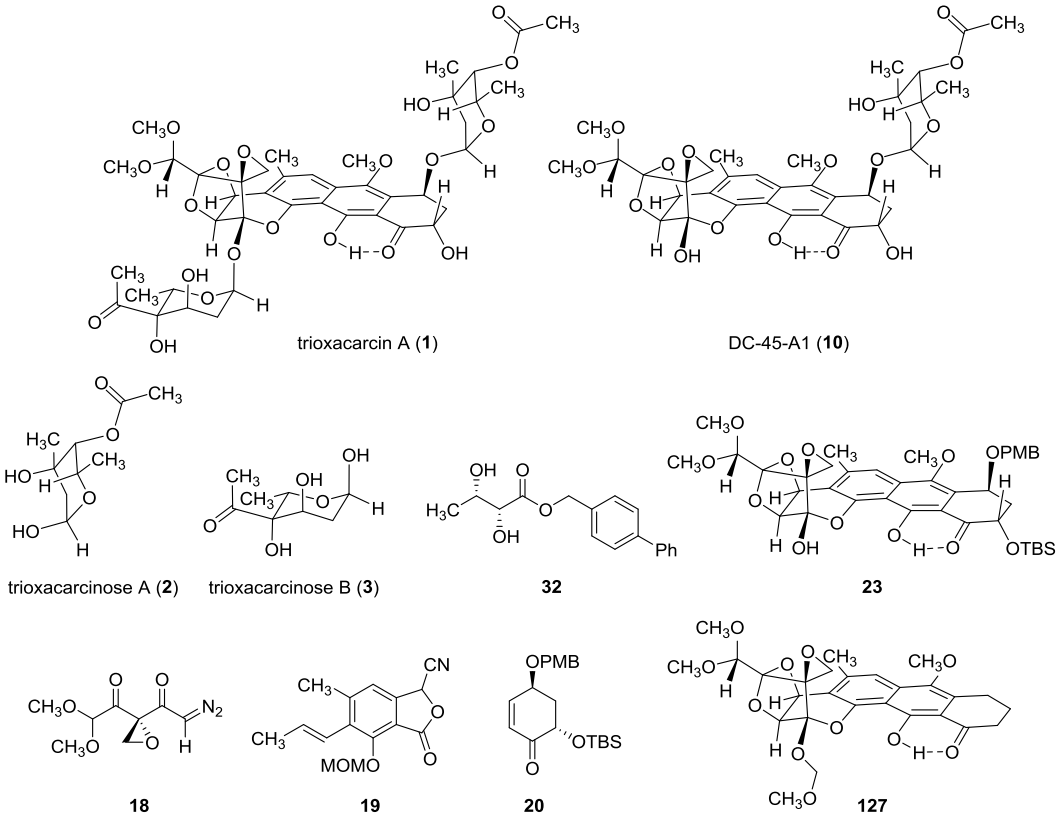
## Abstract

The trioxacarcins are structurally complex, highly oxygenated bacterial isolates that potently inhibit the growth of human cancer cells in culture as a consequence of their ability to alkylate guanosine residues of duplex DNA. This dissertation presents a component-based synthetic route to the trioxacarcin structural class, broadly defined, which resulted in fully synthetic routes to trioxacarcin A (**1**), DC-45-A1 (**10**), and a diverse collection of analogs.

Two efficient synthetic routes were developed to the deoxysugar trioxacarcinose A (**2**) found within most trioxacarcins. Both routes relied on diastereoselective, chelation-controlled additions of allylmetal reagents to free or protected *syn*-α,β-dihydroxyketones. A scalable protocol provided their common syn diol starting material **32**, using a *p*-phenylbenzyl ester as an expedient for purification and enantioenrichment by recrystallization.

Diastereoselective glycosylation reactions were developed for the coupling of activated forms of trioxacarcinose A to fully functionalized trioxacarcin core substrates. This methodology enabled the preparation of the natural product DC-45-A1 (**10**) in three chemical steps from synthetic precursor **23**. Incorporation in tandem of an activated

derivative of trioxacarcinose B (**3**) prepared by postdoctoral researcher Thomas Magauer resulted in an 11-step synthesis of trioxacarcin A (**1**) from precursor **23**.




The synthetic route to trioxacarcin A (**1**) proceeded by the assembly of five components of similar structural complexity: epoxy diazo diketone **18**, cyanophthalide **19**, cyclohexenone **20**, a trioxacarcinose A glycosyl donor, and a trioxacarcinose B glycosyl donor. A diverse collection of trioxacarcin analogs was prepared by variation of these modular components, both individually and in combination, to introduce deep-seated structural modifications which would have been difficult to access by other means. Through measurements of antiproliferative activity in cultured human cancer cells, this program in analog synthesis has begun to define the structural features which enable or disfavor potent growth inhibition. In particular, novel trioxacarcins such as

methoxymethyl aglycon **127** have been identified which retain antiproliferative activity at levels comparable to trioxacarcin A (**1**) despite significant structural simplification.

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## List of Abbreviations

| | |
|---|---|
| A | adenine |
| AcOH | acetic acid |
| aq | aqueous |
| *c* | concentration (g/100 mL) |
| C | cytosine |
| CAM | aqueous ceric ammonium molybdate solution |
| *cis* | *L.*, on the same side |
| $cm^{-1}$ | wavenumber |
| DDQ | 2,3-dichloro-5,6-dicyano-1,4-benzoquinone |
| DIPEA | diisopropylethylamine |
| DMAP | 4-*N,N*-dimethylaminopyridine |
| DMF | *N,N*-dimethylformamide |
| DMSO | dimethyl sulfoxide |
| DNA | deoxyribonucleic acid |
| dr | diastereomeric ratio |
| *E* | *Ger.*, entgegen |
| ee | enantiomeric excess |
| ent- | enantiomeric |
| epi- | epimeric |
| equiv | equivalent |
| ESI | electrospray ionization |
| FTIR | Fourier transform infrared |

| | |
|---|---|
| G | guanine |
| g | gram |
| GI | 50% growth inhibition |
| H460 | human cell line derived from large cell lung cancer cells |
| HPLC | high-pressure liquid chromatography |
| HRMS | high-resolution mass spectrometry |
| Hz | hertz |
| $IC_{70}$ | seventy-percent maximal inhibitory concentration |
| $IC_{50}$ | fifty-percent maximal inhibitory concentration |
| *J* | coupling constant |
| KHMDS | potassium hexamethyldisilazide |
| kg | kilogram |
| K-selectride | potassium tri-*sec*-butylborohydride |
| L | liter |
| LC/MS | liquid chromatography–mass spectrometry |
| $LD_{50}$ | median lethal dose |
| M | molar |
| mg | milligram |
| μg | microgram |
| MHz | megahertz |
| MIC | minimum inhibitory concentration |
| mL | milliliter |
| μL | microliter |

| | |
|---|---|
| μM | micromolar |
| mM | millimolar |
| *m/z* | mass to charge ratio |
| mmol | millimole |
| μmol | micromole |
| MOM | methoxymethyl |
| MS | molecular sieves; mass spectrometry |
| NMR | nuclear magnetic resonance |
| nmol | nanomole |
| P388 | murine lymphocytic leukemia cell line |
| PMB | 4-methoxybenzyl |
| ppm | parts per million |
| rac- | racemic |
| *R* | rectus (Cahn–Ingold–Prelog system) |
| $R_f$ | retention factor |
| RP | reversed phase |
| *S* | sinister (Cahn–Ingold–Prelog system) |
| T | thymine |
| TBAF | tetra-*n*-butylammonium fluoride |
| TBS | *tert*-butyldimethylsilyl |
| THF | tetrahydrofuran |
| TLC | thin-layer chromatography |
| TMEDA | *N,N,N',N'*-tetramethylethylenediamine |

| | |
|---|---|
| TMSCN | cyanotrimethylsilane |
| TMSOTF | trimethylsilyl trifluoromethanesulfonate |
| *trans* | *L.*, across |
| TsOH | *para*-toluenesulfonic acid |
| *Z* | *Ger.*, zusammen |

# Chapter 1

# Introduction

## The Trioxacarcin Class of Natural Products

The trioxacarcins are structurally complex, highly oxygenated bacterial metabolites that broadly inhibit the growth of cultured eukaryotic and bacterial cells. In 1981 Tomita and coworkers[1] reported the isolation of trioxacarcins A (**1**), B (**5**), and C (**6**) (previously referred to as DC-45-A, DC-45-B1, and DC-45-B2, respectively[2]) from an actinomycete bacterial strain, designated as *Streptomyces bottropensis*, found in a soil sample collected in Sapporo, Japan (Figure 1.1). A subsequent patent disclosed the isolation of DC-45-A1 (**10**) and DC-45-A2 (**11**) from the same bacterial strain (Figure 1.2).[3] In 2004 Maskey and coworkers examined extracts from the marine *Streptomyces* sp. isolate B8652 and isolated trioxacarcins A (**1**), B (**5**), and C (**6**) along with the novel trioxacarcins D (**7**), E (**8**), and F (**9**) (Figure 1.1).[4] The latter report also described the isolation of gutingimycin (**4**), a 1:1 covalent adduct of trioxacarcin A (**1**) and guanine (Figure 1.2).[4] Scientists at American Cyanamid Company described the isolation of the closely related LL-D49194 natural products from *Streptomyces vinaceus-drappus*, obtained from a soil sample collected in La Encanada, Peru (Figure 1.2).[5]

All known trioxacarcins share a common aglycon core containing a dihydroanthracenone chromophore which is hydroxylated at positions C2 and C4 (see

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[1] (a) Tomita, F.; Tamaoki, T.; Morimoto, M.; Fujimoto, K. *J. Antibiot.* **1981**, *34*, 1519–1524. (b) Tamaoki,
[2] Tomita, F.; Tamaoki, T.; Shirahata, K.; Iida, T.; Morimoto, M.; Fujimoto, K. Antibiotic Substances DC-45, and Their Use as Medicaments. U.S. Patent 4,511,560, 1985.
[3] Shirahata, K.; Iida, T. Compounds Having Antibiotic Activity, Processes for Their Preparation, Pharmaceutical Compositions Containing Them and Their Use as Medicaments. U.S. Patent 4,459,291, **1984**.
[4] Maskey, R. P.; Helmke, E.; Kayser, O.; Fiebig, H. H.; Maier, A.; Busche, A.; Laatsch, H. *J. Antibiot.* **2004**, *57*, 771–779.
[5] (a) Maiese, W.; Labeda, D.; Korshalla, J.; Kuck, N.; Fantini, A.; Wildey, M.; Thomas, J.; Greenstein, M. *J. Antibiot.* **1990**, *43*, 253–258. (b) Borders, D. B.; Fantini, A. A.; Labeda, D. P.; Lee, M. D.; Maiese, W. M.; Testa, R. T., Antitumor Agents LL-D49194$\alpha_1$, LL-D49194$\beta_1$, LL-D49194$\beta_2$, LL-D49194$\beta_3$, LL-D49194$\gamma$, LL-D49194$\delta$, LL-D49194$\varepsilon$, LL-D49194$\xi$, LL-D49194$\eta$, LL-D49194$\omega_1$, LL-D49194$\omega_2$, and LL-D49194$\omega_3$. U.S. Patent 4,626,503, **1986**.

Figure 1.1 for trioxacarcin numbering). To this chromophore is fused a complex and highly oxygenated motif containing either a spiro epoxide (trioxacarcins A (**1**), C (**6**), and D (**7**)) or the corresponding vicinal diol (trioxacarcins B (**5**), E (**8**), and F (**9**)), along with a C13 hemiacetal, a C16 dimethyl acetal, and either a C15 ketal or (within trioxacarcin F (**9**)) a C15 hemiketal.

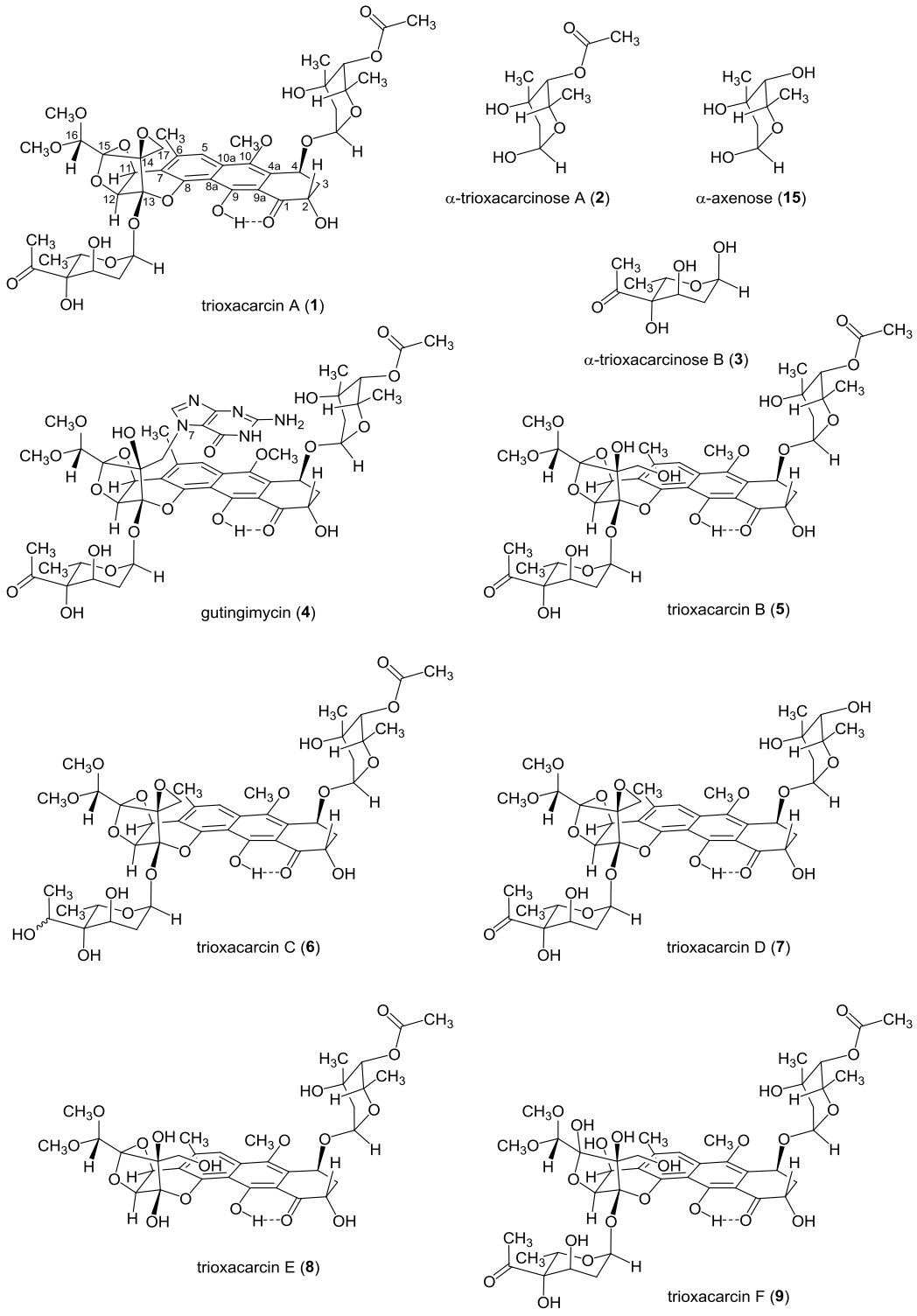


**Figure 1.1.** Structures of Trioxacarcins A (**1**), B (**5**), C (**6**), D (**7**), E (**8**), and F (**9**), Trioxacarcinose A (**2**), Axenose (**15**), Trioxacarcinose B (**3**), and Gutingimycin (**4**).

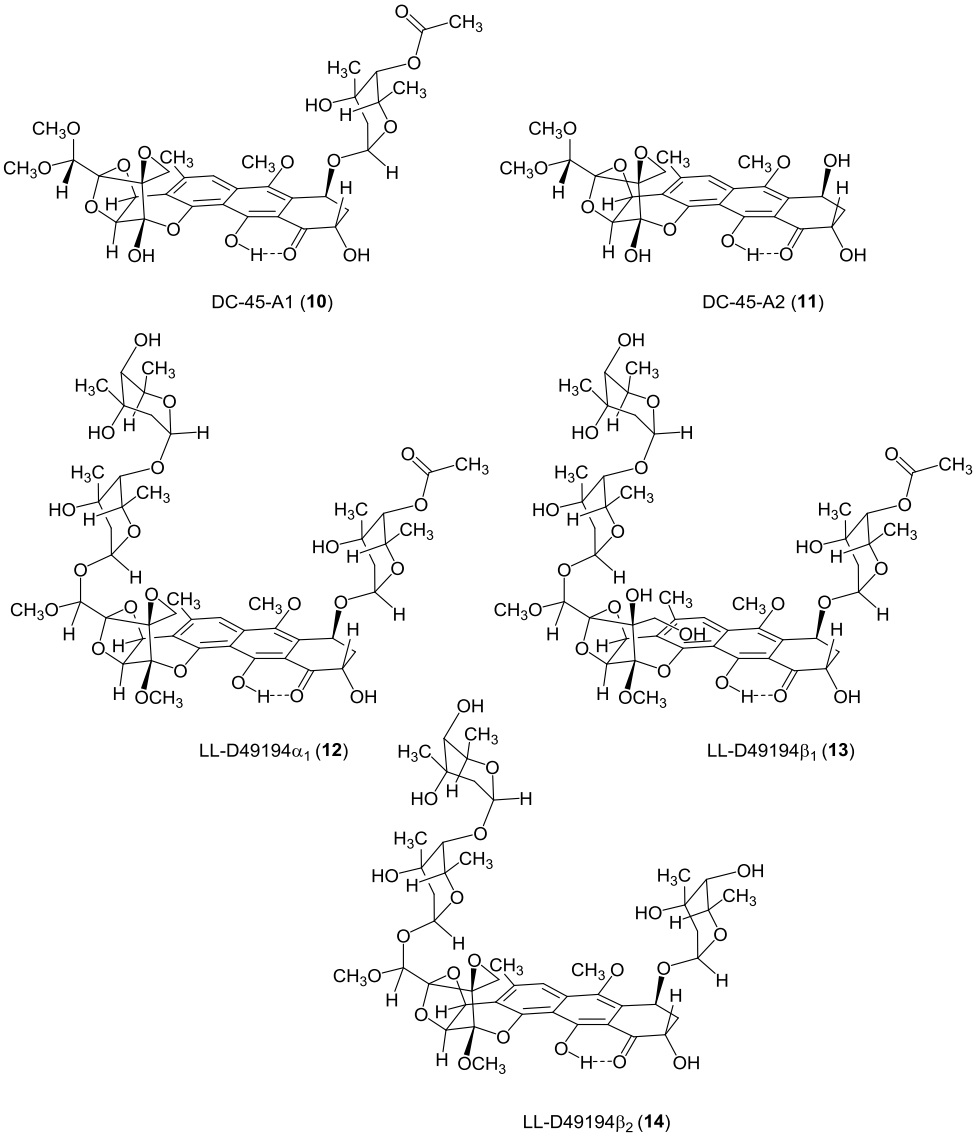


**Figure 1.2.** Structures of the Trioxacarcin Natural Products DC-45-A1 (**10**) and DC-45-A2 (**11**), and Structures of LL-D49194 Natural Products LL-D49194$\alpha_1$ (**12**), LL-D49194$\beta_1$ (**13**), and LL-D49194$\beta_2$ (**14**).

The chemical structure of trioxacarcin A (**1**) was determined by a series of spectroscopic, degradation, and crystallographic studies. Methanolysis of trioxacarcin A (**1**) in 0.1% hydrochloric acid–methanol provided the trioxacarcin aglycon, DC-45-A2 (**11**, also referred to as "trioxacarcinone A") along with two methyl glycosides, both

obtained as mixtures of α- and β-anomers (Scheme 1.1; the α-anomers are depicted).[6] The first such glycosidic component, eponymously named methyl trioxacarcinoside A (**2**), was found to be the 4-*O*-acetylated version of the previously known deoxysugar axenose (**15**). Axenose (**15**) appears in the natural products axenomycin,[7] polyketomycin,[8] and dutomycin,[9] and has been prepared by chemical synthesis.[10] The second glycosidic component was named methyl trioxacarcinoside B (**3**), a sugar also found within the natural product quinocycline B.[11] Permethylation of DC-45-A2 (**11**) provided trimethylated derivative **16**, and oxidative degradation of this product by treatment with potassium permanganate in acetone provided the crystalline derivative **17**, which retained the complex oxygenated core structure in its entirety. The structure and absolute configuration of derivative **17** were established with certainty by X-ray crystallographic analysis.[12] Later crystallographic studies reported the structure of gutingimycin (**4**)[13] and a complex of trioxacarcin A (**1**) covalently bound to DNA,[14] providing additional confirmation of the structure of trioxacarcin A (**1**); these reports are discussed in greater detail later in this chapter.

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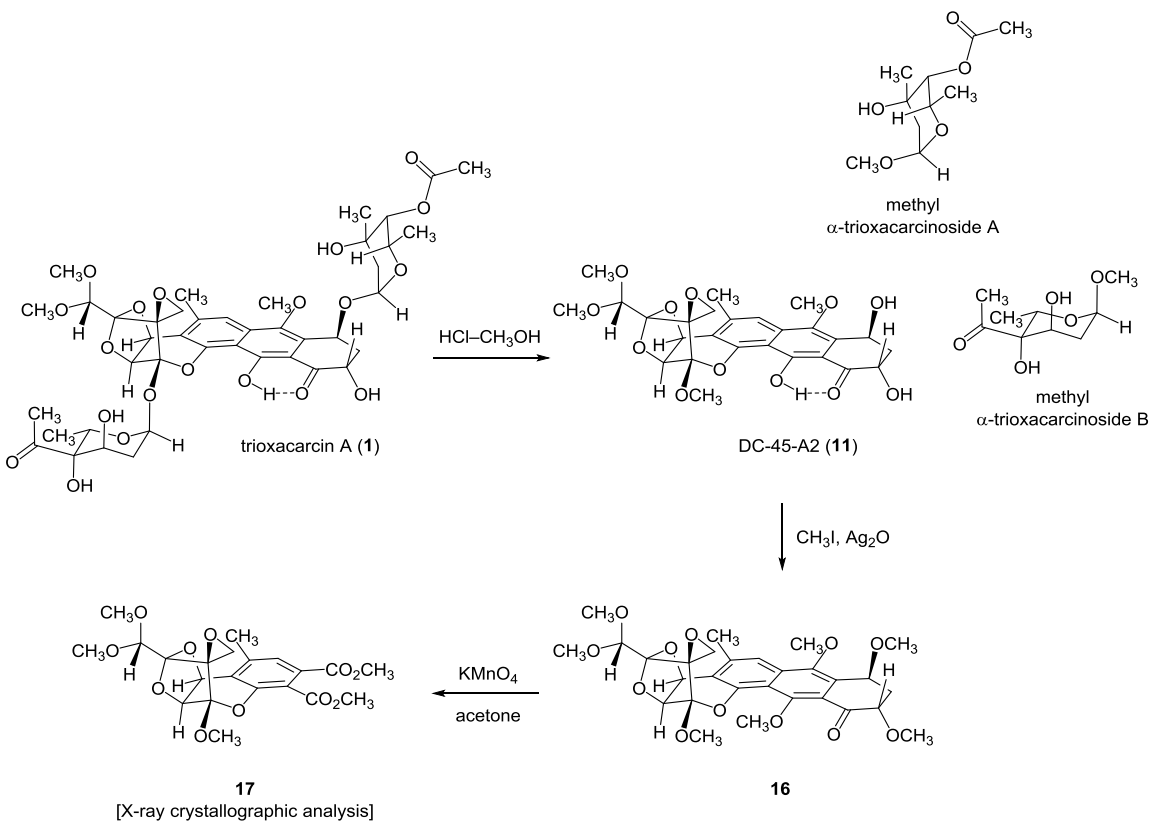
[6] Shirahata, K.; Iida, T.; Hirayama, N. *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu* **1981**, *24*, 199–206.
[7] Arcamone, F.; Barbieri, W.; Franceschi, G.; Penco, S.; Vigevani, A. *J. Am. Chem. Soc.* **1973**, *95*, 2008–2009.
[8] (a) Momose, I.; Chen, W.; Kinoshita, N.; Iinuma, H.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1998**, *51*, 21–25. (b) Momose, I.; Chen, W.; Nakamura, H.; Naganawa, H.; Iinuma, H.; Takeuchi, T. *J. Antibiot.* **1998**, *51*, 26–32.
[9] Xuan, L.-J.; Xu, S.-H.; Zhang, H.-L.; Xu, Y.-M.; Chen, M.-Q. *J. Antibiot.* **1992**, *45*, 1974.
[10] (a) Garegg, P.; Norberg, T. *Acta. Chem. Scand.* **1975**, *29*, 507–512. (b) Giuliano, R. M.; Villani, F. J., Jr. *J. Org. Chem.* **1995**, *60*, 202–211.
[11] Matern, U.; Grisebach, H.; Karl, W.; Achenbach, H. *Eur. J. Biochem.* **1972**, *29*, 1–4.
[12] Hirayama, N.; Iida, T.; Shirahata, K. *Acta Crystallogr. C.* **1989**, *45*, 632–634.
[13] Maskey, R. P.; Sevvana, M.; Usón, I.; Helmke, E.; Laatsch, H. *Angew. Chem. Int. Ed.* **2004**, *43*, 1281–1283.
[14] Pfoh, R.; Laatsch, H.; Sheldrick, G. M. *Nucleic Acids Res.* **2008**, *36*, 3508–3514.




**Scheme 1.1.** Degradation of Trioxacarcin A (**1**) to Provide Methyl α-Trioxacarcinoside A, Methyl α-Trioxacarcinoside B, and Crystalline Derivative **17**.

Like trioxacarcin A (**1**), most natural trioxacarcins are glycosylated, though the number and nature of the glycosidic residues varies. All but two natural trioxacarcins contain trioxacarcinose A (**2**) in an α-linkage with the C4 hydroxyl group, the two exceptions being trioxacarcin D (**7**), which contains α-axenose (**15**), and DC-45-A2 (**11**), which is not glycosylated. Trioxacarcins A (**1**), B (**5**), D (**7**), and F (**9**) contain a trioxacarcinose B (**2**) residue in an α-linkage with the C13 hemiketal function, and trioxacarcin C (**6**) exhibits a reduced form of this sugar, 7-dihydrotrioxacarcinose B.[11] DC-45-A1 (**10**) and DC-45-A2 (**11**) represent 13-deglyco- and 4,13-bis-deglyco-trioxacarcin A (**1**), respectively (Figure 1.2). All known LL-D49194 natural products are trisglycosides, including LL-D49194$\alpha_1$ (**12**), LL-D49194$\beta_1$ (**13**), and LL-D49194$\beta_2$ (**14**)

depicted in Figure 1.2. These molecules contain either trioxacarcinose A (**2**) or axenose (**15**) present at the C4 hydroxyl group and an interesting α(1→4)-linked disaccharide formed from two axenose (**15**) residues linked to the C16 hemiketal (trioxacarcin numbering).

The trioxacarcins were found to exhibit a number of compelling activities in biological systems, the most notable of which is their striking antiproliferative activity in cultured human cancer cell lines. Maskey and coworkers measured the $IC_{70}$ values of trioxacarcins A (**1**), B (**5**), C (**6**), and D (**7**) and gutingimycin in a panel of cancer cell lines, and trioxacarcin A (**1**) in particular exhibited sub-nanomolar $IC_{70}$ values in several cases (Table 1.1).[4] Trioxacarcins C (**6**) and D (**7**), also potent antiproliferative agents, differ from trioxacarcin A (**1**) only in slight modifications to the glycosyl residues, indicating that the precise nature of the glycosyl residues within these compounds may be a key determinant of their biological activity. Trioxacarcins A (**1**), B (**5**), and C (**6**) demonstrated antitumor activity in mice, showing, for example, decreased tumor volume in murine sarcoma 180 and increased life span in murine leukemia P388.[1a,15] A 5-day course of daily injections of 0.10 mg/kg of trioxacarcin A (**1**) resulted in a 35% increased lifespan relative to untreated mice.[1a] The trioxacarcins are nevertheless toxic molecules, and the $LD_{50}$ values of trioxacarcins A (**1**), B (**5**), and C (**6**) in mice were found to be 1 mg/kg, 100 mg/kg, and 2 mg/kg, respectively, as determined by the number of survivors 14 days after a single injection of the trioxacarcin compound.[1a] Trioxacarcins A (**1**) and C (**6**) were also reported to rapidly and potently inhibit polynucleotide biosynthesis,

[15] Fujimoto, K.; Morimoto, M. *J. Antibiot.* **1983**, *36*, 1216–1221.

particularly DNA biosynthesis, in *Bacillus subtilis*, as measured by the rate of uptake of $^{3}$H-labeled precursors.[1b,15] Protein synthesis was inhibited to a much lesser extent.

**Table 1.1.** Antiproliferative Activities of Trioxacarcins in a Panel of Cultured Human Cancer Cells (Adapted from Maskey et al.)[4]

| | cancer cell line* ($IC_{70}$, μM) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **compound** | HT-20 | SF-268 | H-460 | LXFA 526L | LXFL 526L | MCF-7 | MEXF 514L | PC3M | RXF 631L |
| trioxacarcin A | <0.34 | <0.0003 | <0.0003 | <0.34 | <0.0003 | <0.0003 | 0.002 | 0.01 | 0.08 |
| trioxacarcin B | 7.22 | 1.65 | 1.64 | 2.77 | 0.68 | 1.47 | 6.43 | >3.35 | 2.4 |
| trioxacarcin C | <0.34 | 0.01 | <0.0003 | <0.34 | 0.02 | 0.002 | 0.16 | 0.04 | 0.13 |
| trioxacarcin D | 0.87 | 0.18 | 0.04 | <0.36 | 0.77 | 0.08 | 1.57 | 1.86 | 0.76 |
| gutingimycin | >2.96 | >2.96 | >2.96 | >2.96 | 3.34 | >2.96 | >2.96 | >2.96 | >2.96 |

* HT-29 (colon cancer), SF-268 (central nervous system cancer), H-460 (large-cell lung cancer), LXFA 526L (adeno lung cancer), LXFL 526L (large-cell lung cancer), MCF-7 (mammary cancer), MEXF 514L (melanoma), PC3M (prostate cancer), RXF 613 (renal cancer).

The trioxacarcins were also reported to exhibit antibacterial activity, particularly against Gram-positive organisms. As an example, trioxacarcins A (**1**), B (**5**), and C (**6**) exhibited minimum inhibitory concentrations (MICs) of 0.2, 50, and 0.4 μg/mL, respectively, in *Staphylococcus aureus* ATCC 6538P.[1a] Trioxacarcins A (**1**) and D (**7**) were found to potently inhibit the growth of *Plasmodium falciparum*, a causative agent of malaria in humans.[4]

From these biological studies of trioxacarcins a trend also emerges that trioxacarcin B (**5**) and gutingimycin (**4**), both of which lack an epoxide ring, are broadly and substantially less active in biological systems than the other trioxacarcins studied, indicating that an intact epoxide plays a critical role in these activities.

The LL-D49194 natural products are also potent antiproliferative agents, with LL-D49194$\alpha_1$ (**12**), LL-D49194$\beta_1$ (**13**), LL-D49194$\beta_2$ (**14**) all promoting life extension in murine leukemia and melanoma.[5] A phase I study of the most potent of these compounds, LL-D49194$\alpha_1$ (**12**), was conducted in the early 1990s in 15 patients with diverse metastatic cancers that were refractory to existing therapies. Although one patient in this study with colon cancer responded with an improvement in performance that was sustained for six months, a fatality associated with cardiotoxicity led to suspension of the trial. A retrospective analysis suggested that the murine models that were used to determine dosing in the trial predicted human pharmacokinetics poorly; drug exposures more than fourfold higher than anticipated were observed in patients.[16]

Many of the above findings indicated that the biological activity of the trioxacarcins is believed to derive from their interaction with duplex DNA, and the nature of this interaction was elucidated in a series of seminal studies. Maskey and coworkers in 2004 reported the isolation and structural determination of gutingimycin (**4**)[13] by X-ray crystallographic analysis and NMR studies. Within the crystal structure (Figure 1.3), a covalent bond was observed between N7 of the guanine subunit and C17 of the trioxacarcin subunit (see Scheme 1.2 for numbering), as if the spiro epoxide present within trioxacarcin A (**1**) had been subject to nucleophilic opening by the guanine base. The guanine subunit in this solid-state structure is stacked nearly parallel to the plane of the trioxacarcin chromophore, with an interplanar distance of approximately 3.2 Å. The C2 hydroxyl group of the trioxacarcinose B subunit is hydrogen-bonded to the N2 amino group of the guanine subunit (N–H···O). The authors also reported the compelling

[16] Cassidy, J.; Graham, M. A.; Huinink, W. T. B.; McDaniel, C.; Setanoians, A.; Rankin, E. M.; Kerr, D. J.; Kaye, S. B. *Cancer Chemoth. Pharm.* **1993**, *31*, 395–400.

observation that treatment of trioxacarcin A (**1**) with duplex DNA results in the formation of a stable complex, and that heating of this complex at 100 °C results in the liberation of gutingimycin (**4**), presumably as a result of alkylation and depurination of DNA (Scheme 1.2).

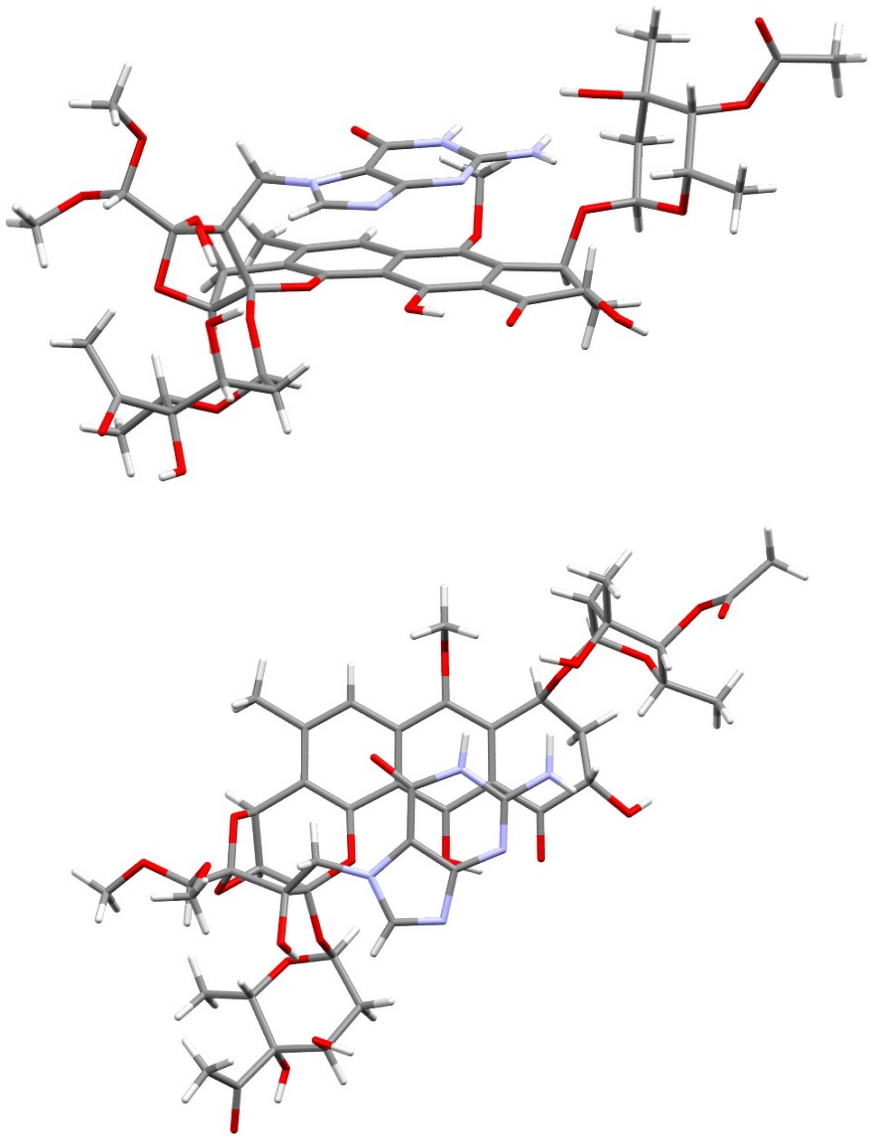
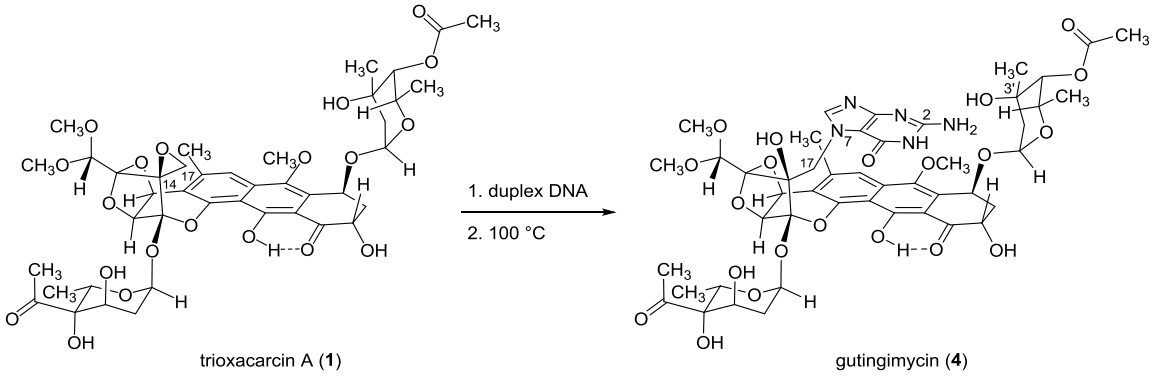


**Figure 1.3.** Two Views of the Crystal Structure of Gutingimycin (**4**)




**Scheme 1.2.** Formation of Gutingimycin (**4**) from Trioxacarcin A (**1**) and Duplex DNA.

Additional insight into the sequence of events involved in the interaction of trioxacarcin A (**1**) with duplex DNA came in the form of crystallographic studies by Pfoh and coworkers, who in 2008 characterized by X-ray crystallography the 2:1 covalent intercalation complex of trioxacarcin A (**1**) with the self-complementary duplex oligonucleotide d(AACCGGTT) to 1.78-Å resolution.[14] Within this structure (Figure 1.4), a covalent bond between C17 of the trioxacarcin and the N7 position of a guanine residue was observed, as in the structure of gutingimycin (**4**). Trioxacarcin A (**1**) has intercalated on the 3' side of the alkylated guanine residue, and the dihydroanthracenone chromophore is oriented nearly parallel to the planes of the base pairs surrounding it, displaying stacking interactions. The complex showed a distorted B-DNA geometry, with the trioxacarcinose A (**2**) residue positioned within the minor groove and the trioxacarcinose B residue (**3**) positioned in the major groove. Interestingly, the intercalation of trioxacarcin A (**1**) displaced the penultimate 3'-thymine residue, which

flipped out of the helix. The adenine with which this flipped-out thymine residue had been base-paired has formed a new pairing interaction with the terminal 3'-thymine.

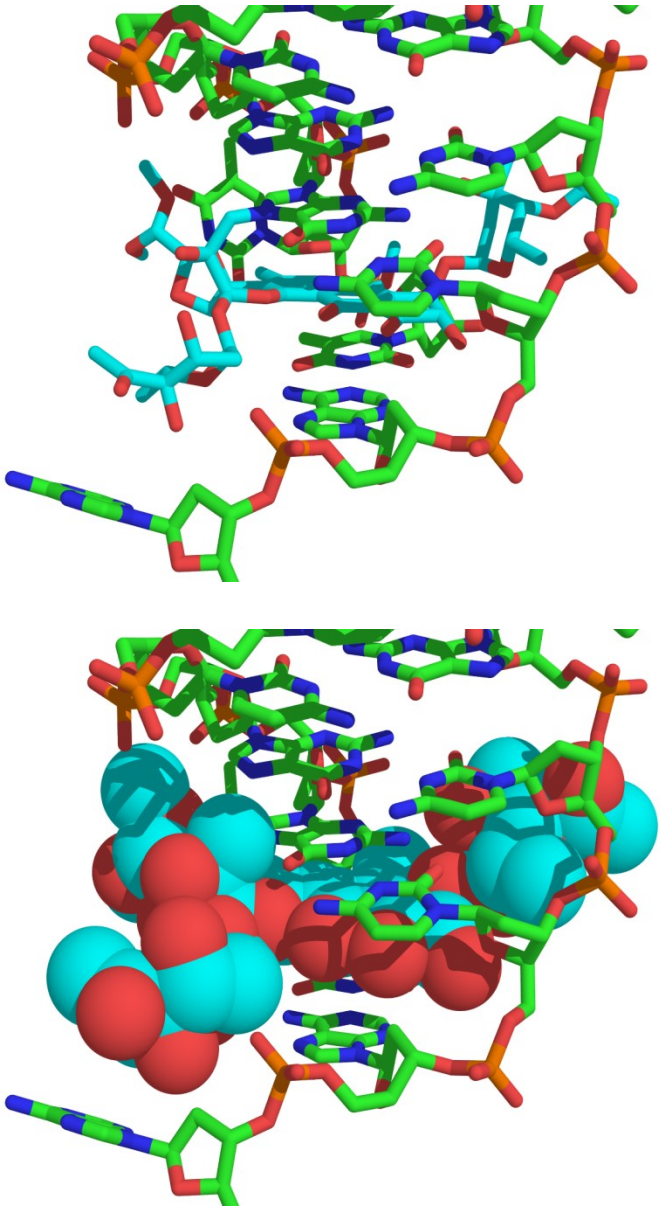


**Figure 1.4.** Two Depictions of the Crystal Structure of Trioxacarcin A (**1**) Covalently Bound to the DNA Duplex d(AACCGGTT).

In order to examine the details of the molecular interaction of trioxacarcin A (**1**) with duplex DNA, Fitzner and coworkers studied the reaction of trioxacarcin A (**1**) with several duplex DNA oligonucleotides using a variety of analytical methods.[17] The authors observed an increase in the melting temperature ($T_m$) of the self-complementary duplex DNA oligonucleotide d(AATTACGTAATT) from $T_m$ = 34 °C to $T_m$ = 41 °C upon treatment with 2 equivalents of trioxacarcin A (**1**). Because a similar increase in melting temperature ($T_m$ = 42 °C) was observed upon treatment of the same oligonucleotide with trioxacarcin B (**5**), which lacks an intact spiro epoxide, it was suggested that the increase in helical stability can be attributed to the intercalation and groove binding of the trioxacarcin, and not to the formation of a covalent complex. Treatment of d(CAATTATAATTG) with trioxacarcin A (**1**), on the other hand, resulted in a small change in melting temperature, from 31 °C to 29 °C, from which the authors concluded that intercalation and/or groove binding are likely not taking place. The covalent adduct of trioxacarcin A (**1**) and d(AATTACGTAATT) was observed by HPLC-MS and found to be stable for more than 24 hours at 23 °C, and upon heating to 80 °C, conversion to gutingimycin (**4**) was observed with first-order kinetics.

In the same study,[17] adduct formation between trioxacarcin A (**1**) and six 12-mer DNAs of varying sequence was observed by HPLC and ESI-MS. The covalent adduct formed to varying extents, with the self-complementary AATTACGTAATT being the most reactive (53% adduct formation) and AATTAGAAATT (not self-complementary) showing almost no conversion to a covalent adduct (1%). These results suggest that double helix formation and the nature of the residues flanking the guanine to be alkylated

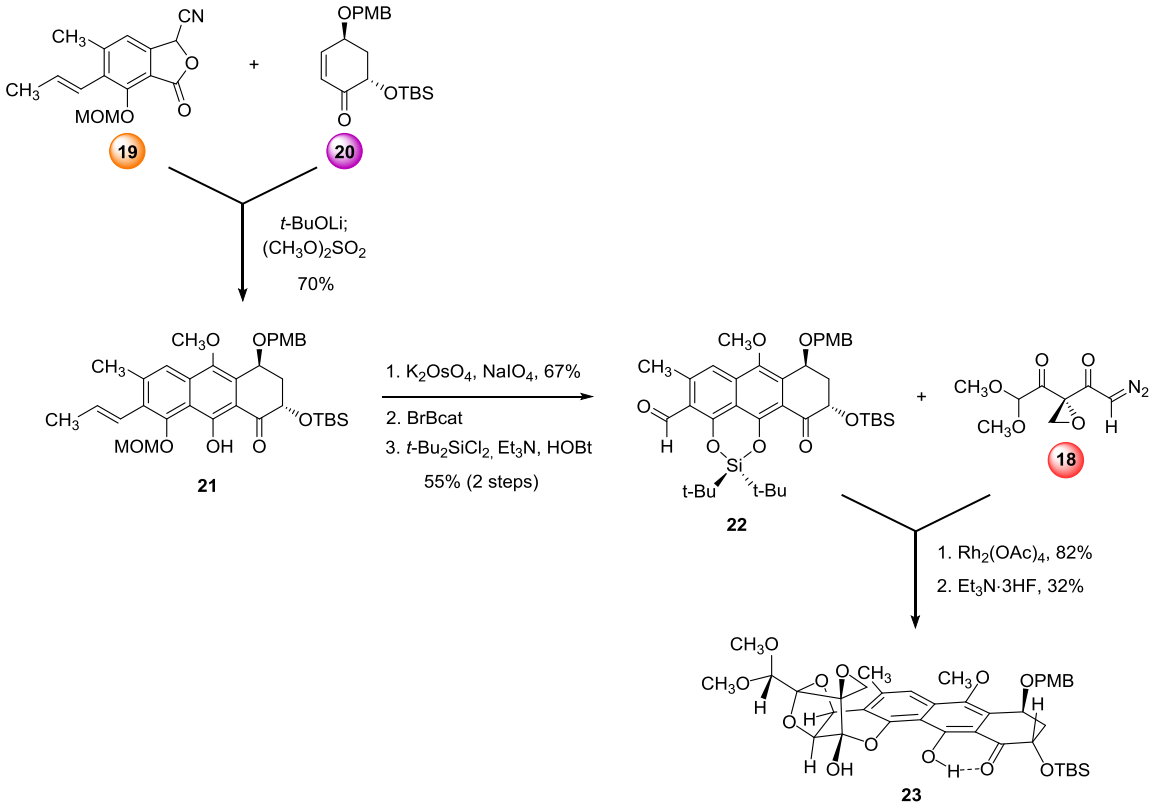
[17] Fitzner, A.; Frauendorf, H.; Laatsch, H.; Diederichsen, U. *Anal. Bioanal. Chem.* **2008**, *390*, 1139–1147.

both influence the trioxacarcin-mediated alkylation of DNA, perhaps by influencing the positioning of the guanine and spiro epoxide reaction partners.

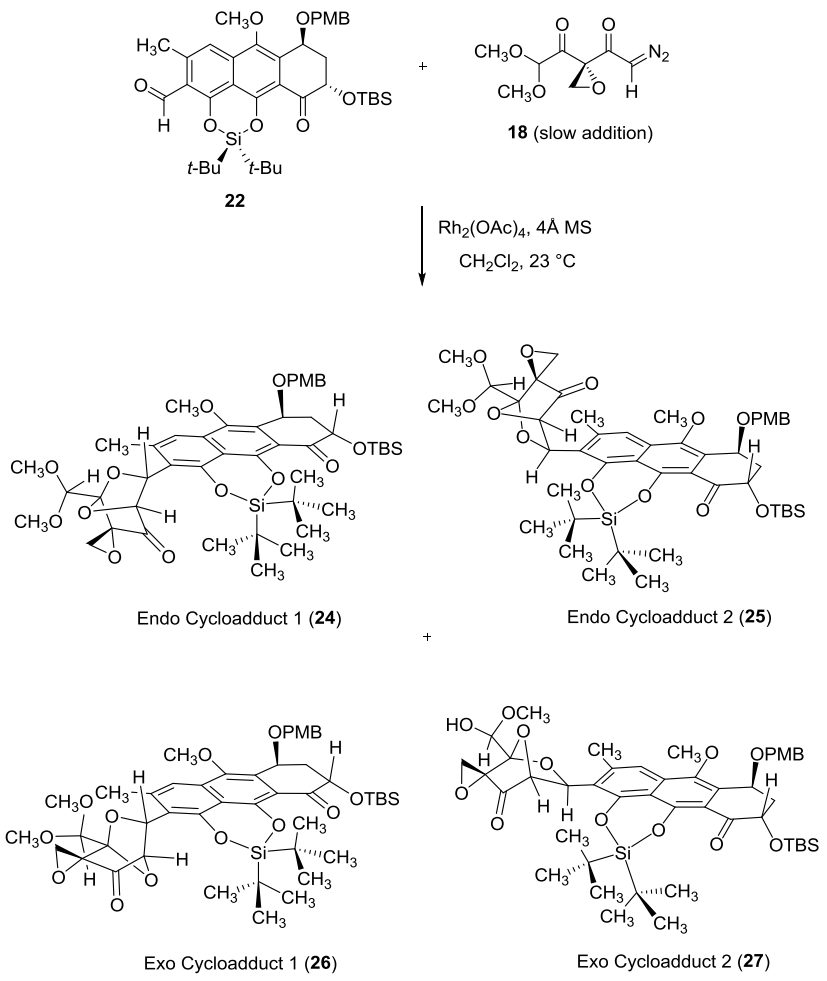
**A Component-Based Synthetic Strategy for Trioxacarcins**

In prior work in the Myers research group at Harvard University, graduate students Jakub Švenda and Nicholas Hill developed a convergent synthetic route to synthetic precursor **23**, a differentially-protected form of DC-45-A2 (**11**), from three components of similar synthetic complexity.[18] The three components—epoxy diazo diketone **18**, cyanophthalide **19**, and cyclohexenone **20**—were assembled in a six-step sequence shown in (Scheme 1.3). Cyanophthalide **19** and cyclohexenone **20** were coupled in an anionic cyclization reaction to provide the full dihydroanthracenone chromophore present within product **21**. A three-step sequence of oxidative cleavage of the disubstituted olefin, removal of the MOM ether protective group, and protection of the revealed bis-phenol function as the corresponding di-*tert*-butyl siloxane provided aldehyde **22**. In a key step, aldehyde **22** underwent a dipolar cycloaddition reaction with a carbonyl ylide intermediate formed from epoxy diazo diketone **18** in the presence of rhodium acetate. This cycloaddition reaction formed a mixture of four diastereomeric cycloadducts, which are depicted in Scheme 1.4. Only the endo diastereomer **25** is stereochemically congruent with the natural trioxacarcins. This mixture was desilylated by brief treatment with triethylamine–trihydrofluoride to provide a mixture of diastereomeric hemiketals, from which synthetic precursor **23** was isolated by RP-HPLC.

[18] Švenda, J.; Hill, N.; Myers, A. G. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 6709–14.




**Scheme 1.3.** Synthesis of Synthetic Precursor **23** From Three Components: Epoxy Diazo Diketone **18**, Cyanophthalide **19**, Cyclohexenone **20** (Labeled with Circled Numbers).




Relative Distribution of Cycloadducts (%)[a]

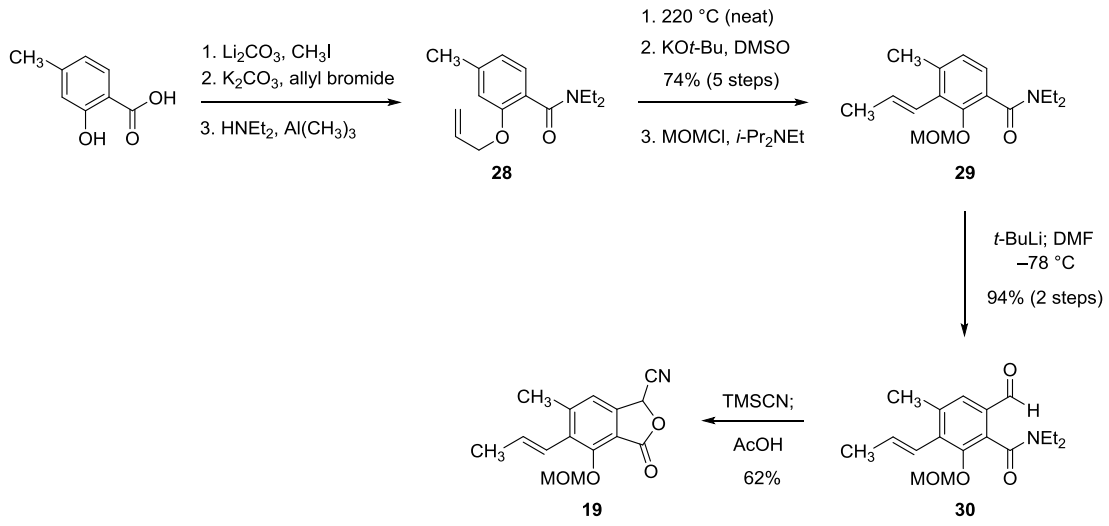
| Endo 1 (**24**) | Endo 2 (**25**) | Exo 1 (**26**) | Exo 2 (**27**) |
|---|---|---|---|
| 44 | **40** | 14 | 2 |

[a] Determined by analysis of the $^1$H NMR spectrum of the crude product mixture

**Scheme 1.4.** Carbonyl Ylide–Aldehyde Cycloaddition between Aldehyde **22** and Epoxy Diazo Diketone **18**.[18]

Cyanophthalide **19** was made available in an efficient, scalable sequence from 4-methylsalicylic acid. This route is reproduced in full in Scheme 1.5 to facilitate discussion in Chapter 4, when modifications to this component are introduced. The

starting material 4-methylsalicylic acid was first converted to allyl ether **28** in a three-step sequence. Claisen rearrangement of **28** followed by alkene isomerization to the internal position and protection of the phenol as the corresponding MOM ether provided arene **29**. Directed *ortho*-lithiation of **29** and trapping of the resulting aryllithium species with DMF furnished aldehyde **30**. Treatment of aldehyde **30** with cyanotrimethylsilane provided an intermediate cyanohydrin (not isolated), which formed cyanophthalide **19** upon stirring in glacial acetic acid.[18]




**Scheme 1.5.** Synthesis of Cyanophthalide **19**.

The diazo diketone component **18** was prepared in an 8-step sequence from 2,2-dimethoxyacetaldehyde, and the preferred route to cyclohexenone building block **20** proceeded in 10 steps using L-malic acid as starting material (Figure 1.5).[18]

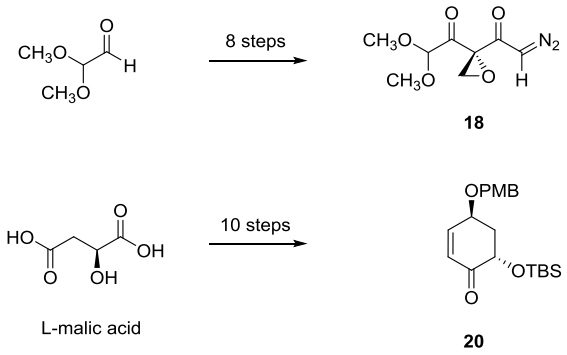



**Figure 1.5.** Starting Materials and Step Counts for Syntheses of Epoxy Diazo Diketone Component **18** and Cyclohexenone Component **20**.[18]

We sought to utilize the differential protection incorporated within synthetic precursor **23** for the selective functionalization of the trioxacarcin aglycon. In particular, we anticipated that this precursor would serve directly as a substrate for the introduction of carbohydrates such as trioxacarcinose B (**2**) by glycosylation of the hemiketal hydroxyl group at position C13. After cleavage of the *p*-methoxybenzyl ether protective group, the precursor would provide a substrate for selective introduction of carbohydrates such as trioxacarcinose A (**3**) by glycosidation of the hydroxyl group liberated at position C4, which we expected to be more reactive. In this manner we sought to prepare natural trioxacarcins such as trioxacarcin A (**1**) by the addition of two remaining modular components (i.e. glycosidic subunits derived from trioxacarcinose A (**2**) and trioxacarcinose B (**3**)) to our convergent route. The result would constitute a synthesis of **1** from five components of similar complexity.

Additionally, we recognized the unique potential for diversification inherent in a convergent, component-based route and envisioned the preparation of trioxacarcin analogs by component variation which would be difficult or impossible to access by other

means. Modification of more than one component would lead to multiplicative expansion of the pool of trioxacarcins (broadly defined) available for study.

In Chapter 2, I describe two practical routes to trioxacarcinose A (**2**) in forms suitable for glycosylation reactions, both proceeding by apparent chelation-controlled additions of allylmetal reagents to α,β-dioxygenated ketones. This work enabled fully synthetic routes to trioxacarcin A (**1**) and DC-45-A1 (**10**) by stereoselective glycosylation reactions, the details of which I present in Chapter 3. In Chapter 4 I describe the results of efforts in trioxacarcin analog synthesis which represent a realization of the potential for diversification inherent in our component-based synthetic route, and in Chapter 5 I present the antiproliferative activities I measured for fully synthetic trioxacarcins, highlighting aspects of the structure-activity relationships which our platform provided a unique ability to study.

# Chapter 2

# Synthesis of Trioxacarcinose A in Forms Suitable for Glycosylation Reactions

## Introduction

In this chapter, I describe the development of two efficient syntheses of the rare deoxysugar trioxacarcinose A (**2**) in forms suitable for glycosidic coupling reactions. After an introduction to the trioxacarcinose A carbohydrate class, I present a scalable synthesis of syn-2,3-dihydroxybutyrate esters in enantiomerically pure form, using a *p*-phenylbenzyl ester group as an expedient for purification and enantioenrichment by recrystallization. The syn-2,3-dihydroxybutyrate esters prepared in this manner provided the starting materials for two synthetic routes to trioxacarcinose A derivatives, both of which utilized the principle of α-chelation control in a diastereoselective addition of an allylmetal reagent to a syn-α,β-dioxygenated ketone substrate. The routes I present enabled the preparation of gram quantities of optically pure trioxacarcinose A derivatives. I conclude this chapter by discussing the preparation of anomerically activated derivatives of this sugar. These anomerically activated derivatives were successfully employed in the syntheses of the glycosylated natural trioxacarcins DC-45-A1 (**10**) and trioxacarcin A (**1**), as described in Chapter 3, as well as in the preparation of numerous trioxacarcin analogs, as described in Chapter 4.

Most known trioxacarcins, including trioxacarcin A (**1**) and DC-45-A1 (**10**), contain the rare, eponymous deoxysugar trioxacarcinose A (**2**) in an α-glycosidic linkage with the C4 hydroxyl group (Figure 1.1, Figure 1.2). The desacetyl form of trioxacarcinose A, known as axenose (**15**), is also naturally occurring and appears within the natural products axenomycin,[7] polyketomycin,[8] and dutomycin.[9] Two synthetic routes

to axenose have appeared previously in the literature.[10,19] Garegg and Norberg prepared axenose in 13 steps (1.6% yield) from L-fucose as starting material,[10a] and Giuliano and Villani prepared axenose in 12 steps (~4% yield) using 2-deoxy-D-ribose as starting material.[10b] For our planned synthetic route to the trioxacarcins we required access to gram quantities of optically pure trioxacarcinose A (**2**) in a form suitable for glycosidic coupling reactions, and we envisioned that an enantioselective synthesis proceeding from non-carbohydrate starting materials was likely to succeed. A retrosynthetic analysis of trioxacarcinose A (**2**) is shown in Figure 2.1. We considered the open-chain form of this sugar and focused on carbonyl addition transforms which would disconnect carbon-carbon bonds to C3. We envisioned that addition of an allylmetal reagent to an appropriately protected syn-2,3-dihydroxy methyl ketone—or, alternatively, addition of a methylmetal reagent to a syn-2,3-dihydroxy allyl ketone—could construct the C3-tertiary alcohol in a diastereoselective fashion. We envisioned the preparation of either ketone from an appropriately protected syn-2,3-dihydroxybutyrate ester.

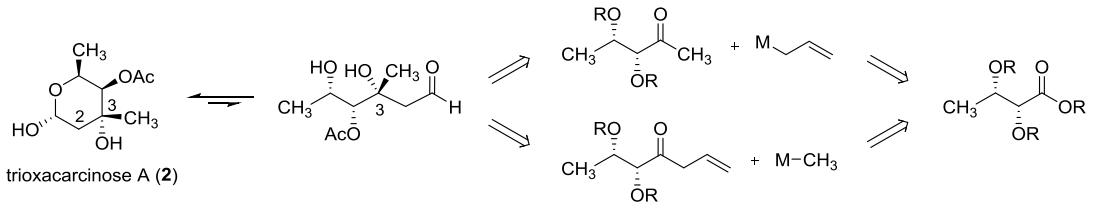


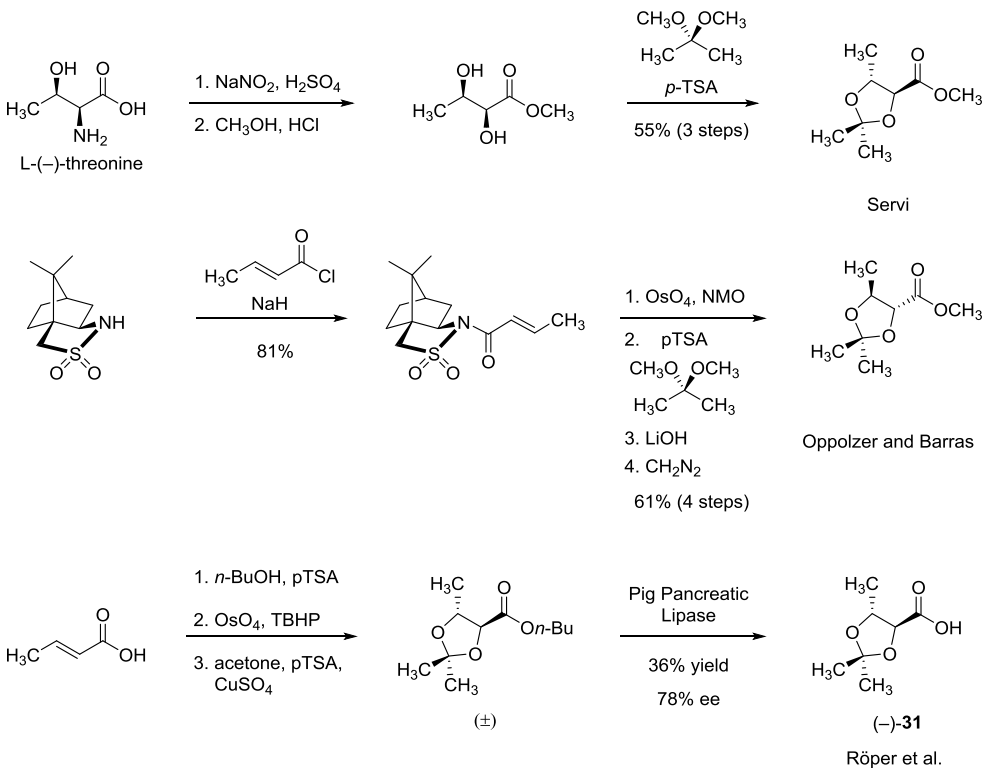

**Figure 2.1.** Retrosynthetic Analysis of Trioxacarcinose A (**2**)

[19] For a synthetic route to 3-*epi*-axenose, see: Roush,W.; Hagadorn, S. *Carbohydr. Res.* **1985**, *136*, 187–193.

**Scalable Synthesis of Enantiomerically Pure syn-2,3-Dihydroxybutyrate**

To prepare trioxacarcinose A (**2**) according to our planned route, we required gram quantities of (2*R*,3*S*)-dihydroxybutyric acid in protected form and targeted (2*R*,3*S*)-dihydroxybutyric acid acetonide ((+)-**31**) as an early intermediate. (2*R*,3*S*)-dihydroxybutyric acid acetonide ((+)-**31**) and its enantiomer have served as valuable building blocks for the assembly of complex carbohydrates and other densely oxygenated molecules.[20] Three prior routes to **31** or its methyl ester had been executed previously, and these are summarized in Scheme 2.1.[21] Servi prepared the methyl ester of (–)-**31** by diazotization–hydrolysis of threonine.[21a] Oppolzer and Barras, on the other hand, prepared the methyl ester of (+)-**31** using an auxiliary-controlled, diastereoselective dihydroxylation reaction.[21b] Finally, Röper and coworkers prepared (–)-**31** by kinetic resolution of the corresponding racemic *n*-butyl ester.[21c] Of these routes, only the diazotization–hydrolysis of threonine[21a] has been used to prepare multigram quantities of **31**, so far as we are aware, and this route is typically used to prepare (–)-**31** from the naturally occurring L-(–)-threonine, as D-(+)-threonine is a less readily available starting material.

[20] (a) Roush, W. R.; Lin, X.; Straub, J. A. *J. Org. Chem.* **1991**, *56*, 1649–1655. (b) Ruiz, M.; Ojea, V.; Ruanova, T. M.; Quintela, J. M. *Tetrahedron: Asymmetr.* **2002**, *13*, 795–799. (c) Gardiner, J. M.; Panchal, N. R.; Stimpson, W. T.; Herbert, J. M.; Ellames, G. J. *Synlett* **2005**, *2005*, 2685–2687.
[21] (a) Servi, S. *J. Org. Chem.* **1985**, *50*, 5865–5867. (b) Oppolzer, W.; Barras, J.-P. *Helv. Chim. Acta* **1987**, *70*, 1666–1675. (c) Pottie, M.; Van der Eycken, J.; Vandewalle, M.; Dewanckele, J.; Röper, H. *Tetrahedron Lett.* **1989**, *30*, 5319–5322.




**Scheme 2.1.** Prior Syntheses of *syn*-2,3-Dihydroxybutyrate and Its Methyl Ester

We considered alternative routes to **31** that would allow for the preparation of large quantities of either enantiomer with equal ease. The Sharpless asymmetric dihydroxylation[22] of a crotonate ester appeared to be an attractive and general solution, potentially providing access to **31** of either enantiomeric series. We noted the precedent established by Ley and coworkers as part of their efforts to synthesize natural products of the bengazole family, in which the dihydroxylation of ethyl crotonate furnished (–)-**31** ethyl ester[23] in 96% yield (>95% ee, 3-g scale). In this methodology, relatively large volumes of organic extractant were used to isolate the highly water-soluble product, and

[22] Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483–2547.

[23] As recently as 2005, literature reports had indicated that **31** ethyl ester was commercially available: Gardiner, J.; Panchal, N.; Stimpson, W.; Herbert, J.; Ellames, G. *Synlett* **2005**, *17*, 2685–2687. However, to the best of our knowledge, this substance is no longer widely available. For the preparation of **31** ethyl ester, see: Sakai, T.; Nakamura, T.; Fukuda, K.; Amano, E.; Utaka, M.; Takeda, A. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 3185–3188.

chromatographic purification was required.[24] In view of this precedent, we began to survey a larger set of crotonate esters and reaction conditions in an attempt to develop a procedure which was optimized toward ease of purification, scalability, and enantiomeric purity of the product.

Methyl, ethyl, and *n*-hexyl crotonate esters are each commercially available in geometrically pure form (reported purity 100% trans). Dihydroxylation of *trans*-methyl crotonate with potassium osmate (0.50 mol %), $(DHQ)_2PHAL$[25] (1.0 mol %), methanesulfonamide (1 equiv), potassium ferricyanide (3 equiv), and potassium carbonate (3 equiv), conditions reported by Sharpless and coworkers,[26] afforded the syn diol in 89% ee[27] and 45% yield (Table 2.1, entry 1). We believe the low yield can be attributed to the poor efficiency of the extraction of the water-soluble product from the aqueous reaction mixture, but more importantly, the enantioselectivity of the transformation was too low for our purposes. Dihydroxylation of *trans*-*n*-hexyl crotonate, also commercially available, afforded an improved yield (88%), but the enantioselectivity of the transformation was still lower than we desired (80% ee, entry 2). The enantioselectivity was slightly higher when $(DHQ)_2AQN$ was used as ligand (entry 3) in place of $(DHQ)_2PHAL$, as expected.[28]

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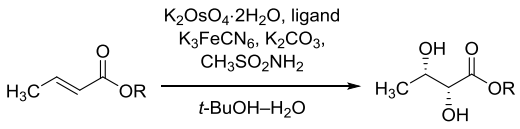
[24] Bull, J. A.; Balskus, E. P.; Horan, R. A.; Langner, M.; Ley, S. V. *Chem.-Eur. J.* **2007**, *13*, 5515–5538.
[25] Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K. S.; Kwong, H. L.; Morikawa, K.; Wang, Z. M. *J. Org. Chem.* **1992**, *57*, 2768–2771.
[26] Kwong, H.-L.; Sorato, C.; Ogino, Y.; Chen, H.; Sharpless, K. B. *Tetrahedron Lett.* **1990**, *31*, 2999–3002. Under these biphasic reaction conditions, the osmium(VI) monoglycolate intermediate cannot be oxidized prior to hydrolysis and release of the diol product. This prevents entry into the so-called "second cycle," which proceeds via an osmium(VI) bisglycolate ester and is poorly enantioselective: Wai, J. S. M.; Marko, I.; Svendsen, J. S.; Finn, M. G.; Jacobsen, E. N.; Sharpless, K. B. *J. Am. Chem. Soc.* **1989**, *111*, 1123–1125.
[27] The enantiomeric excess was determined by $^1H$ NMR analysis of the corresponding bis-Mosher ester. See: Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543–2549.
[28] Becker, H.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **1996**, *35*, 448–451.

**Table 2.1.** Dihydroxylation of *trans*-Crotonate Esters[a]



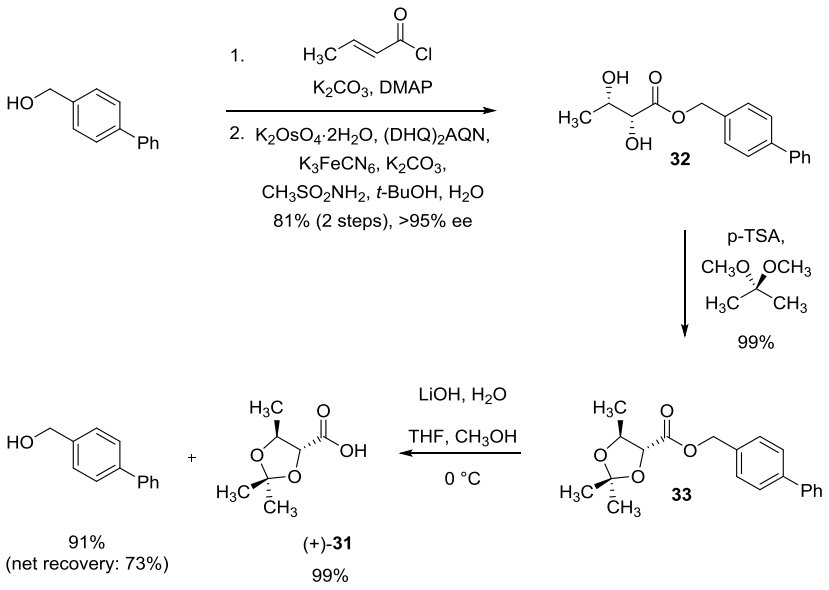

| entry | R | ligand | yield (%) | ee (%)[b] |
|---|---|---|---|---|
| 1 | $CH_3$[c] | $(DHQ)_2PHAL$ | 45 | 89 |
| 2 | $(CH_2)_5CH_3$[c] | $(DHQ)_2PHAL$ | 88 | 80 |
| 3 | $(CH_2)_5CH_3$[c] | $(DHQ)_2AQN$ | 90 | 85 |
| 4 | Bn[d] | $(DHQ)_2AQN$ | 77 | 92 |
| 5 | (*p*-Ph)Bn[d,e] | $(DHQ)_2AQN$ | 81 | 87[f], >95[g] |

[a] Conditions: $K_2OsO_4·2H_2O$ (0.50 mol %), ligand (1.0 mol %), $K_3Fe(CN)_6$ (3 equiv), $K_2CO_3$ (3 equiv), $CH_3SO_2NH_2$ (1 equiv), *t*-BuOH–$H_2O$, 4–23 °C. [b] Determined by integration of the $^1H$ NMR spectrum of the corresponding bis-Mosher ester. [c] The alkene substrate was 100% trans, from a commercial source. [d] The substrate was prepared from commercial crotonoyl chloride (~20:1 E:Z; 90% purity) and was used without purification. [e] 0.25 mol % $K_2OsO_4·2H_2O$, 0.50 mol % $(DHQ)_2AQN$, and 6 equiv $K_3Fe(CN)_6$ were used. [f] Before purification. [g] After a single recrystallization of the product from dichloromethane–hexanes.

In order to improve the reaction further we prepared several crotonate esters by reaction of a number of alcohols and commercial crotonoyl chloride, which is inexpensive but impure (~20:1 mixture of trans and cis isomers, respectively; 90% purity). The unpurified esters were dihydroxylated and the enantiomeric excess was determined by $^1H$ NMR analysis of the corresponding bis-Mosher ester as before. Dihydroxylation of benzyl crotonate prepared in this manner afforded diol of 92% ee (Table 2.1, entry 4).[29] Encouraged by this result, we examined the dihydroxylation of several substituted benzyl crotonate esters (*p*-methoxybenzyl, *p*-nitrobenzyl, benzhydryl, and *p*-phenylbenzyl), and all of these were dihydroxylated to furnish diol products of 87–92% ee. We were especially encouraged to observe that the dihydroxylation of *p*-

[29] This degree of enantioselectivity is in excellent agreement with a previous report: Tagami, K.; Takagi, S.; Shiro, M.; Nagao, Y. *Heterocycles* **1997**, *45*, 1663–1669.

phenylbenzyl crotonate (used without purification) afforded a highly crystalline diol product of 87% ee. A single recrystallization of the diol product from dichloromethane–hexanes provided *p*-phenylbenzyl (2*R*,3*S*)-dihydroxybutyrate (**32**) of >95% ee (entry 5).

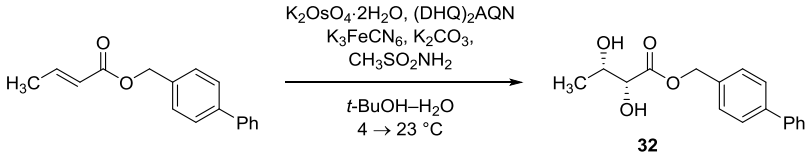



**Scheme 2.2.** Synthesis of Carboxylic Acid (+)-**31**

Dihydroxylation of *p*-phenylbenzyl crotonate was easily conducted on a multigram scale. In one experiment, *p*-phenylbenzyl alcohol (22 g, 119 mmol) was esterified with commercial crotonoyl chloride (~20:1 *E*:*Z*; 90% purity) in the presence of potassium carbonate (2.0 equiv) and a catalytic quantity of 4-(dimethylamino)pyridine (DMAP, 10 mol %) (Scheme 2.2). The crude ester, a ~17:1 mixture of *E* and *Z* isomers, was subjected to conditions for the Sharpless asymmetric dihydroxylation. Complete conversion to the diol was achieved with low catalyst loading (0.25 mol % potassium osmate, 0.50 mol % $(DHQ)_2AQN$), an extended reaction time (~5 days), and distributed

addition of potassium ferricyanide (6 equiv).[30] Methanesulfonamide was removed by a sequence of aqueous washes.[31] The unpurified diol product was found to be of 87% ee by $^1$H NMR analysis of the corresponding bis-Mosher ester. One recrystallization of the crude product from dichloromethane–hexanes provided pure diol (+)-**32** of >95% ee in 81% yield from *p*-phenylbenzyl alcohol (Table 2.2, entry 1, "method A"; 27.8-g batch; $[\alpha]_D = +22.7°$ (*c* 1.04, $CH_2Cl_2$)).

**Table 2.2.** Protocols for Large-Scale Dihydroxylation




**32**

| entry | method | $K_2OsO_4·2H_2O$ (mol %) | $(DHQ)_2AQN$ (mol %) | $K_3Fe(CN)_6$ (equiv) | time at 4 °C | time | yield[b] (%) | ee[c] (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | A | 0.25 | 0.50 | 6 | 2 d | at 23 °C 3 d | 81 | >95 |
| 2 | B | 1.0 | 1.0 | 3 | 30 min | 25 h | 60 | >95 |

[a] The starting material, *p*-phenylbenzyl crotonate, was prepared from commercially available crotonoyl chloride (~20:1 *E*:*Z*; 90% purity) and used without purification. Reactions were performed on a scale of at least 0.1 mol. [b] Isolated yield of pure product, after recrystallization. [c] Determined by integration of the $^1$H NMR spectrum of the corresponding bis-Mosher ester.

In light of the long reaction time required for the optimized dihydroxylation procedure (~5 days), referred to as "method A," (Table 2.2, entry 1) we briefly investigated changes which would shorten the reaction time. More rapid warming of the

[30] As the alkene was a ~17:1 mixture of *E* and *Z* isomers, we expected the dihydroxylation reaction to afford a mixture of syn and anti diols. Syn and anti methyl 2,3-dihydroxybutyrate exhibit a characteristic difference in the chemical shift of the C4 methyl group. $^1$H NMR (300 MHz, CDCl3): anti δ 1.18 (d, 3H, *J* = 6 Hz): Mahalingam, S. M.; Sathyamurthi, N.; Aidhen, I. S. *Adv. Synth. Catal.* **2005**, *347*, 715–717; syn δ 1.31 (d, 3H, *J* = 6.5 Hz): Umemura, E.; Tsuchiya, T.; Umezawa, S. *J. Antibiot.* **1988**, *41*, 530–537. On the basis we estimate that product was formed as a ~21:1 mixture of syn and anti diastereomers, respectively. The minor diastereomer was removed upon recrystallization.

[31] Similar washings of diol product mixtures derived from more water-soluble esters (such as methyl) led to significant loss of product.

reaction mixture (under otherwise identical conditions, 0.1 mol scale) led to poorer conversion, and *p*-phenylbenzyl alcohol was observed in the crude product mixture. The latter byproduct presumably formed as a result of saponification of either the diol product (**32**) or the substrate under the moderately basic aqueous reaction conditions.[32] Sharpless and coworkers have noted that for electron-poor olefins turnover can be increased with little or no diminution in enantioselectivity by increasing the loading of osmium.[33] Using increased catalyst loading (1.0 mol% potassium osmate, 1.0 mol % $(DHQ)_2AQN$) and adding sodium bicarbonate (3 equiv) to buffer the aqueous phase, the large-scale dihydroxylation reaction was complete within 25 h. The crude product mixture contained ≤3% *p*-phenylbenzyl alcohol, as determined by integration of its $^1$H NMR spectrum. Recrystallization of this crude product from dichloromethane–hexanes provided pure diol **32** in 60% yield and >95% ee (Table 2.2, entry 2, "method B"). We believe that this protocol, with a shorter reaction time and a lower yield, provides a viable alternative to the longer reaction time but higher yield obtained using method A.

With efficient procedures for the preparation of enantiomerically pure diol (+)-**32**, we found that a short sequence of standard transformations led to the desired dihydroxybutyric acid acetonide (+)-**31** (Scheme 2.2). Protection of the diol function was accomplished in quantitative yield by stirring with 2,2-dimethoxypropane and *p*-toluenesulfonic acid monohydrate (5 mol %) to provide acetonide **33**. Saponification in

[32] Saponification appears to be a background process under all conditions examined for the large-scale dihydroxylation of 4-phenylbenzyl crotonate. Thus, using "method A" (0.25 mol % $K_2OsO_4 \cdot 2H_2O$, 5-day reaction time), the saponification product comprised ~3% of the crude mixture, as determined by integration of a diagnostic signal in the $^1$H NMR spectrum (500 MHz, CDCl3): δ 4.75 (s, 2H, Ar–C*H*$_2$OH).

[33] (a) Walsh, P. J.; Sharpless, K. B. *Synlett* **1993**, 605–606. (b) AD-mix containing 5 mol % potassium osmate and 1 mol % PHAL ligand is referred to in the literature as "Modified-AD-mix". See: Bennani, Y. L.; Sharpless, K. B. *Tetrahedron Lett.* **1993**, *34*, 2079–2082. (c) Roberts et al. have designed a "Super-AD-mix" containing 8–10 mol % osmium tetroxide and 10 mol % PHAL ligand: Hermitage, S. A.; Murphy, A.; Nielsen, P.; and Roberts, S. M. *Tetrahedron* **1998**, *54*, 13185–13202.

aqueous lithium hydroxide furnished the desired carboxylic acid (+)-**31** in 99% yield after extractive isolation. In the largest run, this route furnished 15 g of enantiomerically pure acid (+)-**31** in 4 steps and 79% overall yield. Additionally, *p*-phenylbenzyl alcohol, which functioned as an expedient for purification and enantioenrichment by recrystallization, was recovered in 73% overall yield and could be used in subsequent runs.

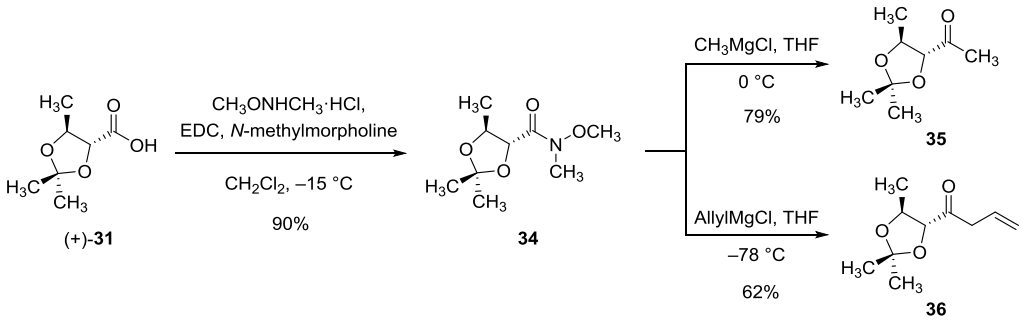
**Synthesis of Methyl Trioxacarcinoside A**

With a practical protocol for preparing carboxylic acid (+)-**31** on a multigram scale, we turned our attention to the conversion of this material to trioxacarcinose A (**2**). We hoped to form the C3 tertiary alcohol of trioxacarcinose A in a diastereoselective fashion by addition of an appropriate allylmetal reagent to a methyl ketone derived from carboxylic acid **31** (or diol **32**), or, alternatively, by addition of an appropriate methylmetal reagent to an allyl ketone derived from the same material (see the retrosynthetic analysis in Figure 2.1). We expected that the choice of diol protective group(s) would influence the stereochemical outcome of organometallic additions to such ketones. It was initially unclear whether the acetonide protective group present within carboxylic acid **31** would be optimal for the diastereoselective additions we envisioned, as the literature did not offer clear guidance as to which protective groups might ensure the desired stereochemical outcome. While α-chelation-controlled[34] additions of Grignard reagents to substrates containing 2-tetrahydrofuranyl and α-benzyloxymethyloxy ketones

[34] (a) Cram, D. J.; Kopecky, K. R. *J. Am. Chem. Soc.* **1959**, *81*, 2748–2755. (b) Still, W. C.; McDonald III, J. H. *Tetrahedron Lett.* **1980**, *21*, 1031–1034. (c) Reetz, M. T. *Acc. Chem. Res.* **1993**, *26*, 462–468. (d) Mengel, A.; Reiser, O. *Chem. Rev.* **1999**, *99*, 1191–1224. (e) Reetz, M. T. *Angew. Chem. Int. Ed.* **1984**, *23*, 556–569.

have been featured in landmark syntheses of natural products,[35] Grignard additions to acyclic ketones bearing both α- and β-oxygenated substituents are of a less certain stereochemical outcome.[36]

To prepare ketone substrates for initial studies of organometallic addition reactions, we first prepared the corresponding Weinreb amide **34** in excellent yield by stirring (+)-**31** with *N*,*O*-dimethylhydroxylamine, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), and *N*-methylmorpholine (17.4-g batch, Scheme 2.3).[37] Weinreb amide **34** was then converted to both the methyl ketone **35**[38] and the allyl ketone **36** by reaction with methylmagnesium chloride and allylmagnesium chloride, respectively, in THF as solvent.



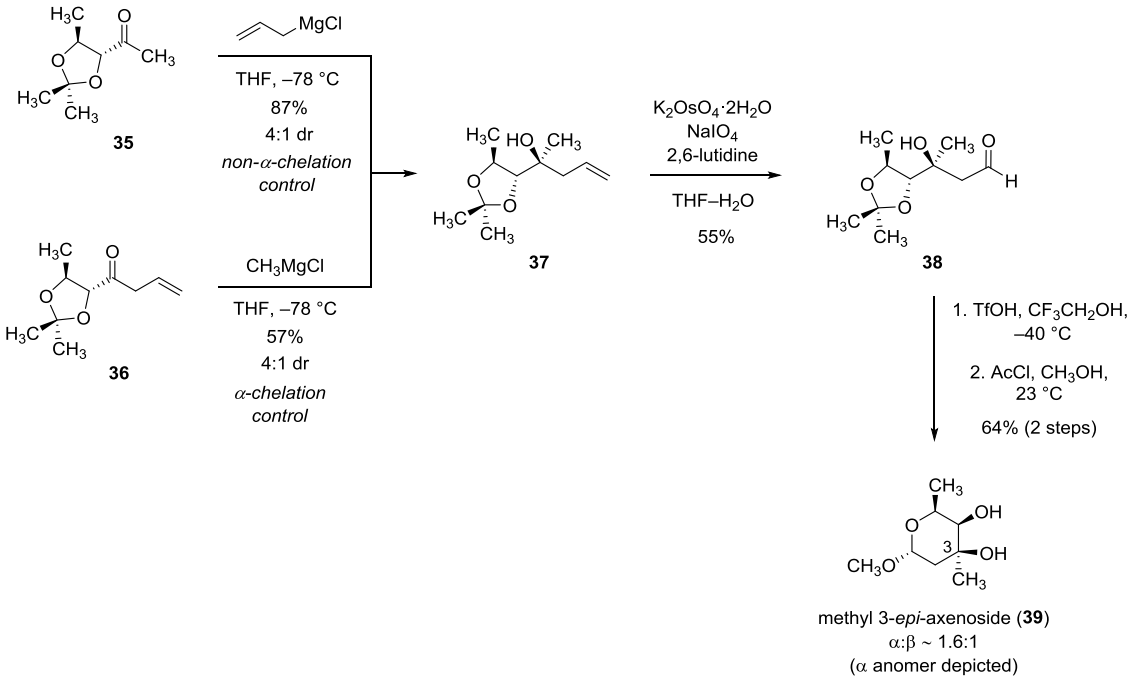

**Scheme 2.3.** Synthesis of Ketones **35** and **36** from Carboxylic Acid **31** via Weinreb Amide **34**.

[35] (a) Nakata, T.; Kishi, Y. *Tetrahedron Lett.* **1978**, *19*, 2745–2748. (b) Collum, D. B.; McDonald III, J. H.; Still, W. C. *J. Am. Chem. Soc.* **1980**, *102*, 2118–2120.
[36] (a) Mulzer, J.; Angermann, A. *Tetrahedron Lett.* **1983**, *24*, 2843–2846. (b) Mead, K.; Macdonald, T. L. *J. Org. Chem.* **1985**, *50*, 422–424.
[37] (a) Campbell, A. D.; Raynham, T. M.; Taylor, R. J. *Synthesis* **1998**, 1707–1709. (b) Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815–3818.
[38] The methyl ketone **35** is volatile, especially at reduced pressure. For this reason the crude product, estimated to be of ~90% purity, was used in subsequent transformations without purification.

We briefly explored additions of Grignard reagents to acetonide-protected methyl ketone **35** and allyl ketone **36** as summarized in Scheme 2.4; however, in neither case was the stereochemical outcome favorable for the purpose of synthesizing trioxacarcinose A (**2**). In one experiment, addition of allylmagnesium chloride to methyl ketone **35** furnished predominantly the stereoisomer **37** (as the major component of a 4:1 mixture of epimers), which is not congruent with trioxacarcinose A at position C3, established by conversion of the adduct **37** to methyl 3-*epi*-axenoside (**39**) by a three-step sequence (Scheme 2.4). In this sequence, the terminal olefin of **37** was cleaved oxidatively to furnish aldehyde **38**, and the acetonide within this product was cleaved by treatment with a catalytic quantity of triflic acid in 2,2,2-trifluoroethanol as solvent at –40 °C.[39] The resulting 2,2,2-trifluoroethyl glycoside was converted to the methyl glycoside **39** by treatment with methanolic hydrochloric acid (formed *in situ* by addition of acetyl chloride to methanol), and the $^1$H NMR spectrum of the major product was in agreement with that of methyl 3-*epi*-axenoside.[19] The stereochemical course of allylmagnesium chloride addition to ketone **35** is therefore consistent with either "polar Felkin–Anh"[40] or β-chelation-controlled transition states, but not with α-chelation-controlled addition. We also observed that addition of methylmagnesium chloride to allyl ketone **36** also provided the same epimer of tertiary alcohol **37** as the major product, which in this case is consistent with an α-chelation-controlled addition. There have been reported instances in which the stereochemical outcomes of allyl and methyl Grignard reagents to the same

[39] Kobayashi, S.; Reddy, R. S.; Sugiura, Y.; Sasaki, D.; Miyagawa, N.; Hirama, M. *J. Am. Chem. Soc.* **2001**, *123*, 2887–2888.
[40] Anh, N. T., Regio- and Stereo-Selectivities in Some Nucleophilic Reactions. In *Organic Chemistry Syntheses and Reactivity*, Springer Berlin Heidelberg: 1980; Vol. 88, pp 145–162.

ketone substrate have been different.[41] We briefly surveyed other achiral allylmetal additions to **35** and methylmetal additions to **36**, but none of the conditions examined produced the desired stereochemical outcome.

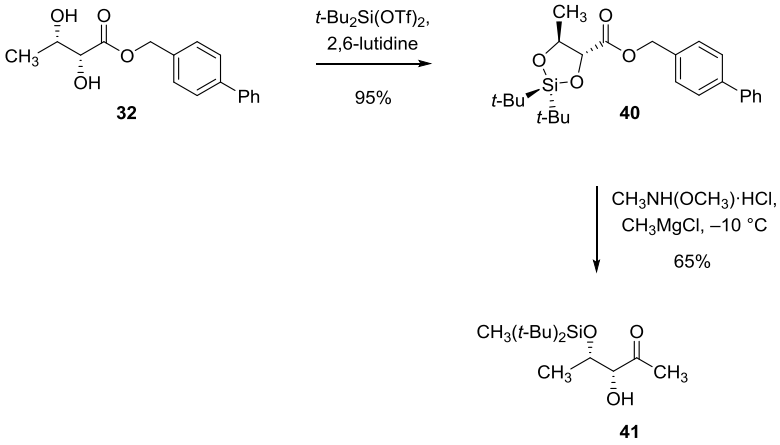



**Scheme 2.4.** Additions to Acetonide-Protected *syn*-2,3-Dihydroxyketones

Due to the unfavorable stereochemical outcomes of addition reactions to the acetonide-protected ketone substrates **35** and **36**, we were led to explore the use of other protective groups for the diol function of diol ester **32**. Accordingly, we prepared the corresponding di-*tert*-butyl siloxane derivative[42] **40** (95% yield, 12.7 g, Scheme 2.5). Fortuitously, upon attempted transformation of the di-*tert*-butylsiloxane ester **40** into the corresponding methyl ketone using the Merck single step process (via the Weinreb amide

[41] Carda, M.; González, F.; Rodríguez, S.; Marco, J. A. *Tetrahedron: Asymm.* **1993**, *4*, 1799–1802.
[42] (a) Trost, B. M.; Caldwell, C. G. *Tetrahedron Lett.* **1981**, *22*, 4999–5002. (b) Corey, E.; Hopkins, P. B. *Tetrahedron Lett.* **1982**, *23*, 4871–4874.

derivative),[37b,43] concomitant, regioselective cleavage of the cyclic siloxane group occurred, giving rise to the di-*tert*-butylmethyl silyl ether **41** (5.3 g, 65% yield). Although regioselective openings of di-*tert*-butylsiloxane derivatives with organolithium reagents have been described,[44] we are unaware of previous examples of the corresponding transformations with Grignard reagents.

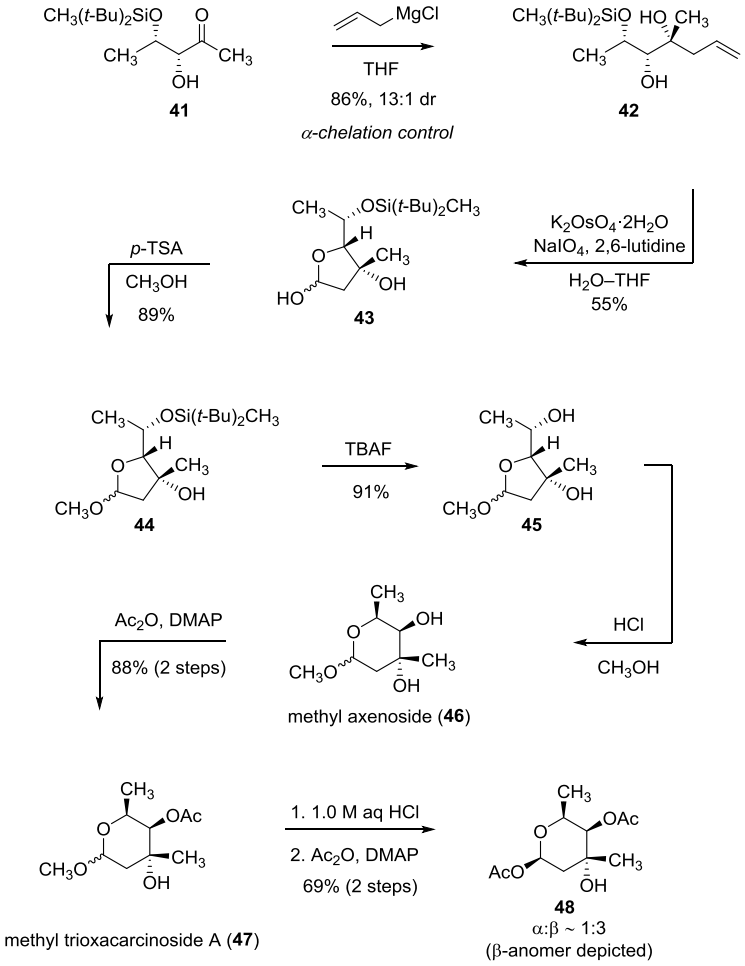



**Scheme 2.5**. Synthesis of Methyl Ketone **41**

The conversion of cyclic siloxane **40** to the monosilyl ether **41** enabled our first route to trioxacarcinose A (Scheme 2.6). Treatment of α-hydroxyketone **41** with allylmagnesium chloride in THF at –78 °C provided tertiary alcohol **42** with 13:1 diastereoselectivity, consistent with an α-chelation-controlled addition mechanism (3.59

[43] Williams, J. M.; Jobson, R. B.; Yasuda, N.; Marchesini, G.; Dolling, U.-H.; Grabowski, E. J. *Tetrahedron Lett.* **1995**, *36*, 5461–5464.
[44] (a) Mukaiyama, T.; Shiina, I.; Kimura, K.; Akiyama, Y.; Iwadare, H. *Chem. Lett.* **1995**, *1995*, 229–230. (b) Tanino, K.; Shimizu, T.; Kuwahara, M.; Kuwajima, I. *J. Org. Chem.* **1998**, *63*, 2422–2423.

g, 86% yield).[45] The product **42** is stereochemically congruent with trioxacarcinose A (**2**), as established by its conversion to methyl axenoside (**46**), en route to **2**.




**Scheme 2.6.** Syntheses of Methyl Axenoside (**46**) and Methyl Trioxacarcinoside A (**47**)

Oxidative cleavage of the terminal alkene of **42** occurred in the presence of potassium osmate and sodium metaperiodate,[46] providing the furanose derivative **43** as a mixture of anomers (1.97 g, 55% yield, α:β ≈ 1:5). Deprotection of the di-*tert*-

[45] Chelation-controlled Grignard additions of α-hydroxyketones, while relatively uncommon, are known. For an example, see: Matsunaga, N.; Kaku, T.; Ojida, A.; Tasaka, A. *Tetrahedron: Asymm.* **2004**, *15*, 2021–2028.
[46] Yu, W.; Mei, Y.; Kang, Y.; Hua, Z.; Jin, Z. *Org. Lett.* **2004**, *6*, 3217–3219.

butylmethyl silyl ether, a robust and hindered protective group,[47] was best accomplished by a two-step sequence. The cyclic hemiacetal **43** was first converted to the more stable methyl glycoside **44** with *p*-toluenesulfonic acid (*p*-TSA) in methanol (89% yield). The methyl glycoside was then smoothly desilylated with TBAF at 23 °C to provide the mixture of methyl furanoside anomers **45** (890 mg, 91% yield). The methyl furanosides **45** were isomerized to the more stable methyl pyranosides **46** (methyl α- and β-axenoside) by a known transformation with methanolic HCl at 23 °C.[10b,48] Analytical data for **46** were in agreement with those previously reported for methyl axenoside.[10] Selective acetylation of the secondary hydroxyl group provided methyl trioxacarcinoside A (**47**, 970 mg, 88% over two steps). Analytical data were in agreement with those reported for the same substance derived from natural sources.[6]

Hydrolysis of methyl trioxacarcinoside A (**47**) in 1.0 M aqueous hydrochloric acid provided trioxacarcinose A itself (**2**), which in turn was acetylated at the anomeric position to provide the 1-*O*-acetyl glycoside **48** in 69% yield over two steps (α:β ≈ 1:3). 1-*O*-acetyl glycosides are known to be effective glycosyl donors, do not require activation for coupling,[49] and can be converted into several other types of glycosyl donors.[50] Our synthetic route provided this anomerically activated form of

[47] Nicolaou, K.; Yue, E. W.; La Greca, S.; Nadin, A.; Yang, Z.; Leresche, J. E.; Tsuri, T.; Naniwa, Y.; de Riccardis, F. *Chem.-Eur. J.* **1995**, *1*, 467–494.

[48] The product is a (presumably, thermodynamic) mixture of methyl pyranoside and furanoside isomers: β-pyranoside: 62%, α-pyranoside: 34%, β-furanoside: 4%, α-furanoside: <1%.

[49] Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1503–1531. (a) Ogawa, T.; Kitajima, T.; Nukada, T. *Carbohyd. Res.* **1983**, *123*, C8–C11. (b) Hayashi, M.; Hashimoto, S.-i.; Noyori, R. *Chem. Lett.* **1984**, 1747–1750. (c) Kihlberg, J. O.; Leigh, D. A.; Bundle, D. R. *J. Org. Chem.* **1990**, *55*, 2860–2863. (d) Sarkar, S.; Lombardo, S. A.; Herner, D. N.; Talan, R. S.; Wall, K. A.; Sucheck, S. J. *J. Am. Chem. Soc.* **2010**, *132*, 17236–17246.

[50] (a) Ogawa, T.; Kitajima, T.; Nukada, T. *Carbohyd. Res.* **1983**, *123*, C8–C11. (b) Hayashi, M.; Hashimoto, S.-i.; Noyori, R. *Chem Lett* **1984**, 1747–1750. (c) Kihlberg, J. O.; Leigh, D. A.; Bundle, D. R. *J. Org. Chem.* **1990**, *55*, 2860–2863. (d) Sarkar, S.; Lombardo, S. A.; Herner, D. N.; Talan, R. S.; Wall, K. A.; Sucheck, S. J. *J. Am. Chem. Soc.* **2010**, *132*, 17236–17246.

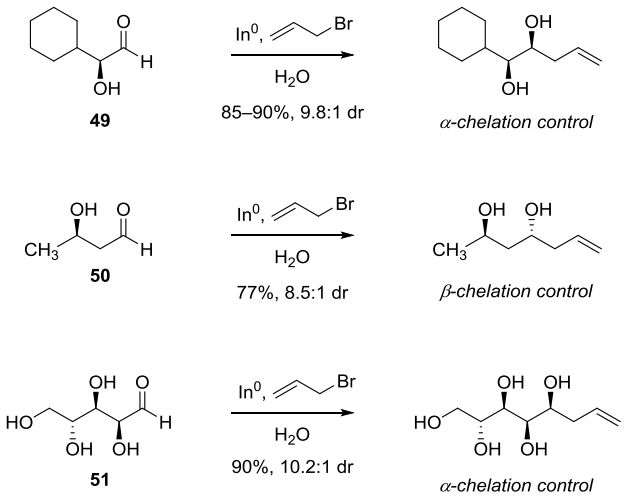
trioxacarcinose A in 12 steps from *p*-phenylbenzyl alcohol in 12% overall yield (84% average yield per step).

**Second-Generation Synthesis of Trioxacarcinose A in Activated Form**

The route outlined above proved to be an effective means of synthesizing trioxacarcinose A (**2**) and derivatives thereof. Nevertheless, we considered an even shorter sequence based on remarkable work from Paquette and coworkers describing diastereoselective, indium-mediated allylation reactions of aldehyde and ketone substrates containing free hydroxyl groups, in water as solvent.[51] Selected examples from the work of Paquette are summarized in Scheme 2.7. For example, Paquette and Mitzel demonstrated that indium-mediated allylation of the α-hydroxyaldehyde **49** proceeded with high diastereoselectivity to afford predominantly the syn product, which is consistent with an α-chelation-controlled addition mechanism.[51a] Allylation of β-hydroxyaldehyde **50** under the same conditions furnished the anti product, consistent with a β-chelation-controlled mechanism. They also reported the very interesting case of In-mediated allylation of the polyol D-arabinose (**51**), for which the directing effects of the α- and β-hydroxyl groups were, in principle, nonreinforcing (and the effects of the γ- and δ-hydroxyl groups were unknown). In this case, the product of α-chelation control was formed with high diastereoselectivity. This result in particular suggested that allylation of the dihydroxy ketone (3*R*,4*S*)-dihydroxy-2-pentanone (**52**), with nonreinforcing α- and β-

[51] (a) Paquette, L. A.; Mitzel, T. M. *Tetrahedron Lett.* **1995**, *36*, 6863–6866. (b) Paquette, L. A.; Mitzel, T. M. *J. Am. Chem. Soc.* **1996**, *118*, 1931–1937. (c) Paquette, L. A.; Lobben, P. C. *J. Org. Chem.* **1998**, *63*, 5604–5616.

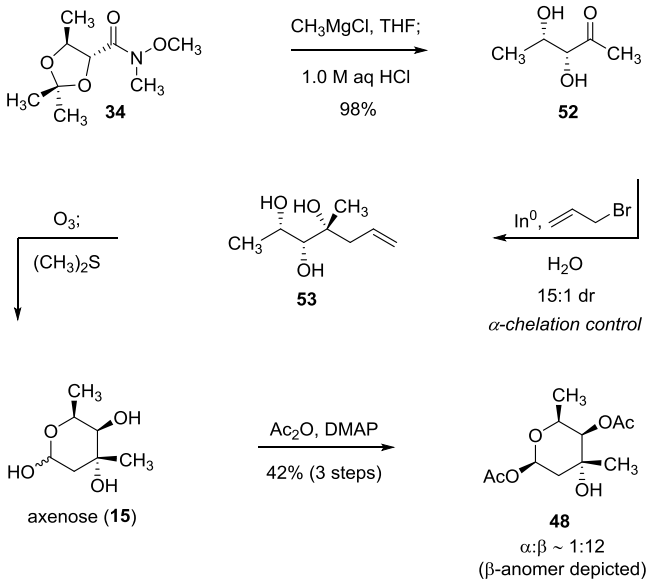
directing effects, might proceed in the desired sense (with α-chelation control) to provide an extraordinarily short and practical sequence to trioxacarcinose A.




**Scheme 2.7.** Indium-Mediated Allylations of Hydroxyaldehydes as Described by Paquette and Mitzel.[51a]

To investigate the proposed indium-mediated allylation reaction, the Weinreb amide **34** (see Scheme 2.3) was first converted to the required dihydroxyketone substrate **52** (9.89 g, 98% yield) in a single operation (Scheme 2.8). Allylation of ketone **52** (3.43 g) using indium powder (1.6 equiv) and allyl bromide (1.6 equiv) in water at 23 °C, conditions typical of those employed by Paquette and Mitzel,[51b] indeed proceeded with predominant α-chelation control to provide the water-soluble triol **53** as a 15:1 mixture of diastereomers. Direct ozonolysis of this diastereomeric mixture followed by reductive quenching with dimethylsulfide provided axenose (**15**), predominantly in its pyranose form. The crude product was acetylated to provide 1-*O*-acetyl trioxacarcinose A (**48**) in

pure form after chromatographic purification (42% yield over three steps from **52**, 2.97 g, α:β ≈ 1:12).

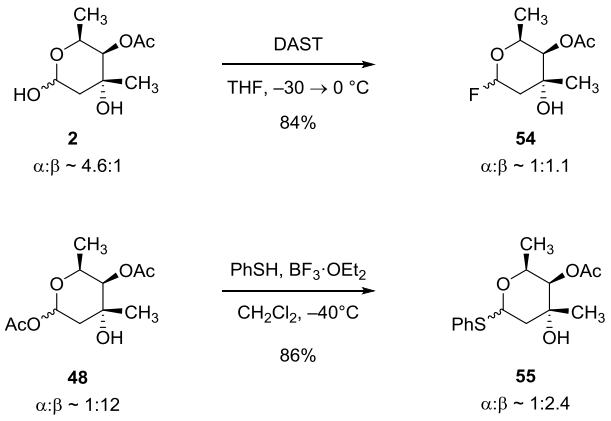



**Scheme 2.8.** Second-Generation Synthesis of 1-*O*-Acetyl Trioxacarcinose A (**48**)

Methanolysis (using acetyl chloride in methanol) of 1-*O*-acetyl trioxacarcinose A (**48**) synthesized by this second route provided methyl trioxacarcinoside A (**47**, 74% yield, see Experimental Section), which provided analytical data indistinguishable from those of **47** from the first route, described above. This second route provided 1-*O*-acetyl trioxacarcinose A (**48**) in 9 steps from *p*-phenylbenzyl alcohol, in 29% overall yield (average 87% yield per step). By virtue of its shorter step count, higher overall yield, and greater convenience, this route provides a particularly useful means to access anomerically activated forms of trioxacarcinose A (**2**).

**Synthesis of Anomerically Activated Derivatives of Trioxacarcinose A**

Fully synthetic 1-*O*-acetyltrioxacarcinose A (**48**), which was first prepared using the first-generation route to trioxacarcinose A (Scheme 2.6) and specifically targeted in the second-generation route (Scheme 2.8), provided one very useful anomerically activated derivative of this sugar. We also briefly explored the preparation of other anomerically activated derivatives of trioxacarcinose A (Scheme 2.9). Addition of diethylaminosulfur trifluoride (DAST) to a solution of trioxacarcinose A (**2**) (prepared by acidic hydrolysis of the methyl glycoside **47**, Scheme 2.6) in tetrahydrofuran at –30 °C afforded the moisture- and acid-sensitive glycosyl fluoride **54** (22 mg, 84%). Analysis of the $^{1}H$ and $^{19}F$ NMR spectra of the crude reaction product revealed that the α- and β-anomers were present in nearly equal amounts (α:β ≈ 1:1.1). The 1-phenylthio derivatives **55** were prepared by treatment of 1-*O*-acetyltrioxacarcinose A (**48**) with thiophenol in the presence of boron trifluoride etherate (103 mg, 86%, α:β ≈ 1:2.4).




**Scheme 2.9.** Preparation of Anomerically Activated Derivatives of Trioxacarcinose A.

## Conclusion

In this chapter, I have described two efficient, enantioselective synthetic routes to the axenose–trioxacarcinose A carbohydrate class from non-carbohydrate starting materials. Both routes relied upon diastereoselective addition reactions of allylmetal reagents to α,β-dioxygenated ketones, using the principle of α-chelation to control the stereochemical outcomes of these additions. The synthetic routes were enabled by a scalable protocol that I developed for the synthesis of their common starting material, the crystalline substance *p*-phenylbenzyl (2*R*,3*S*)-dihydroxybutyrate (**32**). Both routes allowed me to prepare gram quantities of optically pure trioxacarcinose A (**2**) in forms suitable for glycosidic coupling reactions.

In Chapter 3, I describe a component-based synthetic route to the trioxacarcin natural products trioxacarcin A (**1**) and DC-45-A1 (**10**), both of which contain a trioxacarcinose A residue in an α-linkage to the aglycon core. The anomerically activated derivatives 1-*O*-acetyl trioxacarcinose A (**48**) and 1-phenylthiotrioxacarcinoside A (**55**) were used for the introduction of this sugar to synthetic precursors of DC-45-A1 (**10**) and trioxacarcin A (**1**), respectively. Both of these anomerically activated derivatives also proved useful for the introduction of the trioxacarcinose A residue to several fully synthetic trioxacarcin analogs, the preparation of which I describe in Chapter 4.

## Experimental Section

*General Experimental Procedures*

All reactions were performed in round-bottom flasks fitted with rubber septa under a positive pressure of argon, unless otherwise noted. Air- and moisture-sensitive liquids were transferred by syringe or stainless steel cannula. Organic solutions were concentrated by rotary evaporation (house vacuum, ca. 25−40 Torr) at ambient temperature, unless otherwise noted. Analytical thin-layer chromatography (TLC) was performed using glass plates pre-coated with silica gel (0.25 mm, 60 Å pore-size, 230−400 mesh, Merck KGA) impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light, then were stained by submersion in aqueous ceric ammonium molybdate (CAM) or an aqueous sodium hydroxide–potassium carbonate solution of potassium permanganate ($KMnO_4$) followed by brief heating on a hot plate. Flash-column chromatography was performed as described by Still et al.,[52] employing silica gel (60 Å, 32-63 μm, standard grade, Dynamic Adsorbents, Inc.).

*Materials*

Commercial solvents and reagents were used as received with the following exceptions. Tetrahydrofuran, dichloromethane, benzene, toluene, and ether were purified by the method of Pangborn et al.[53] 4-Å molecular sieves (powder, Aldrich) were stored in

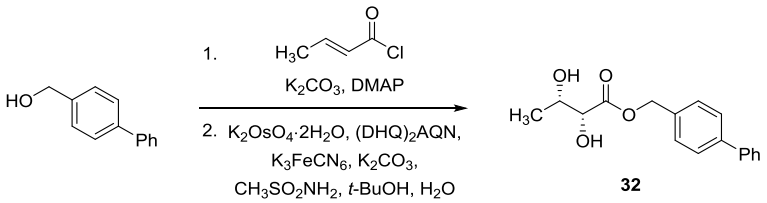
[52] Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.
[53] Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518–1520.

a laboratory oven at 140 °C and were allowed to cool in a desiccator immediately before use.

*Instrumentation*

Proton nuclear magnetic resonance ($^1$H NMR) spectra were recorded using Varian INOVA 500 (500 MHz) or Varian INOVA 600 (600 MHz) NMR spectrometers at 23 °C. Proton chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to residual protium in the NMR solvent ($CHCl_3$, δ 7.26 ppm; DMSO-$d_5$, δ 2.50 ppm). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances), integration, coupling constant (*J*) in Hertz. Carbon nuclear magnetic resonance ($^{13}$C NMR) spectra were recorded using Varian Mercury 400 (100 MHz) or Varian INOVA 500 (125 MHz) NMR spectrometers at 23 °C. Carbon chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to the carbon resonances of the NMR solvent ($CDCl_3$, δ 77.0, DMSO-$d_6$, δ 39.52 ppm). Infrared (IR) spectra were obtained using a Shimadzu 8400S FT-IR spectrometer. Data are represented as follows: frequency of absorption ($cm^{-1}$), intensity of absorption (vs = very strong, s = strong, m = medium, w = weak, br = broad). Optical rotations were measured on a Jasco DIP-0181 digital polarimeter with a sodium lamp and are reported as follows: $[\alpha]_{\lambda}^{T[^\circ C]}$ (c = g/100 mL, solvent). High-resolution mass spectra were obtained at the Harvard University Mass Spectrometry Facility. Melting points were measured using a Thomas Hoover uni-melt apparatus (6427F10).

*(For clarity, intermediates that have not been assigned numbers in the text are numbered sequentially in the Experimental Section beginning with* **56***.)*




(2*R*,3*S*)-Biphenyl-4-ylmethyl 2,3-Dihydroxybutanoate (**32**).

**Method A.**

4-Dimethylaminopyridine (1.46 g, 11.9 mmol, 0.1 equiv) was added to a suspension of crotonoyl chloride (technical grade, 14.0 mL, 146 mmol, 1.22 equiv), *p*-phenylbenzyl alcohol (22.0 g, 119 mmol, 1 equiv), and potassium carbonate (33.0 g, 239 mmol, 2.0 equiv) in dichloromethane (240 mL) at 23 °C. After 7 h, a second portion of crotonoyl chloride (12.7 mL, 133 mmol, 1.11 equiv) was added. After 15 h, water (200 mL) was added. The layers were separated. The aqueous layer was extracted with dichloromethane (200 mL). The organic layers were combined. The combined solution was washed sequentially with saturated aqueous sodium bicarbonate solution (200 mL), 1.0 M aqueous hydrochloric acid solution (200 mL), water (200 mL), then saturated aqueous sodium chloride solution (200 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was dissolved in a mixture of *tert*-butanol (240 mL) and water (240 mL). Potassium carbonate (49.5 g, 358 mmol, 3.0 equiv), potassium ferricyanide (118 g, 358 mmol, 3.0 equiv), and $(DHQ)_2AQN$ (511 mg, 0.596 mmol, 0.005 equiv) were added in sequence, and the mixture was cooled to 4 °C. Potassium osmate dihydrate (110 mg, 0.928 mmol, 0.0025 equiv) was added. After 10 min, methanesulfonamide (5.67 g, 59.6 mmol, 0.5 equiv) was added. After 48 h, the cooling bath was removed and the reaction flask was allowed to warm to 23 °C. After 23 h, a second portion of potassium

ferricyanide (59.0 g, 179 mmol, 1.5 equiv) was added. After 18 h, a third portion of potassium ferricyanide (59.0 g, 179 mmol, 1.5 equiv) was added. After 25 h, the reaction flask was cooled on an ice bath. Sodium sulfite (150 g, 1.19 mol, 10 equiv) was added slowly. After 1 h, the product mixture was extracted with ethyl acetate (6 × 500 mL). The organic layers were combined. The combined solution was washed sequentially with 1.0 M aqueous sodium hydroxide solution (1.5 L) then saturated aqueous sodium chloride solution (1.0 L) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was dissolved in a minimal amount of hot dichloromethane (~200 mL, 35 °C). The resulting solution was heated to boiling in an oil bath at 65 °C, and hexanes was added slowly such that boiling was maintained. Upon the first appearance of a white precipitate, an amount of dichloromethane sufficient to dissolve the precipitate was added (~20 mL). The oil bath was removed and the flask was allowed to cool to 23 °C. The flask was further cooled to –20 °C. After 15 h, a light-yellow crystalline solid had precipitated from the yellow supernatant. The mixture was filtered and the solid was washed with cold hexanes (100 mL, –20 °C) to provide the product, (2*R*,3*S*)-biphenyl-4-ylmethyl 2,3-dihydroxybutanoate (**32**), as light-yellow needles (27.8 g, 81% yield based on *p*-phenylbenzyl alcohol).

To determine the enantiomeric excess, (*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl chloride (6.3 μL, 34 μmol, 2.4 equiv) was added to a solution of (2*R*,3*S*)-biphenyl-4-ylmethyl 2,3-dihydroxybutanoate **32** (4 mg, 14 μmol, 1 equiv) and 4-dimethylaminopyridine (8.2 mg, 67 μmol, 4.8 equiv) in dichloromethane (280 μL) at 23 °C. After 90 min, the product solution was partitioned between ethyl acetate (20 mL) and

water (5 mL). The layers were separated. The organic layer was washed sequentially with 1.0 M aqueous hydrochloric acid solution (5 mL), saturated aqueous sodium bicarbonate solution (5 mL), water (5 mL), then saturated aqueous sodium chloride solution (5 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. Analysis of the $^1$H NMR spectrum of the crude product established that complete conversion to the bis-Mosher ester had occurred. The product was determined to be of 49:1 d.r. ($^1$H NMR analysis (500 MHz, $CDCl_3$): δ 5.76 (qd, 1H, $J$ = 6.8, 2.4 Hz (2*S*,3*R*, minor)), 5.70 (qd, 1H, $J$ = 6.4, 2.0 Hz (2*R*,3*S*, major))), from which we concluded that the starting diol, (2*R*,3*S*)-biphenyl-4-ylmethyl 2,3-dihydroxybutanoate (**2**), was of >95% ee.

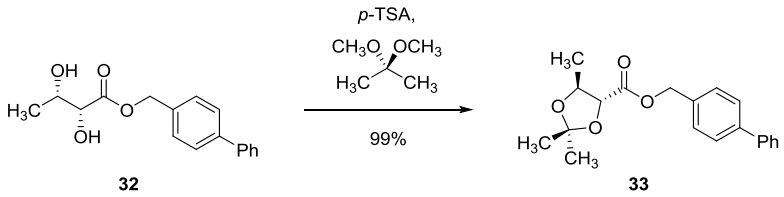
**Method B.**

4-Dimethylaminopyridine (1.46 g, 11.9 mmol, 0.1 equiv) was added to a suspension of crotonoyl chloride (technical grade, 34.3 mL, 358 mmol, 3.0 equiv), *p*-phenylbenzyl alcohol (22.0 g, 119 mmol, 1 equiv), and potassium carbonate (33.0 g, 239 mmol, 2.0 equiv) in dichloromethane (240 mL) at 23 °C. After 12 h, water (200 mL) was added. The layers were separated. The aqueous layer was extracted with dichloromethane (200 mL). The organic layers were combined. The combined solution was washed sequentially with saturated aqueous sodium bicarbonate solution (200 mL), 1.0 M aqueous hydrochloric acid solution (200 mL), water (200 mL), then saturated aqueous sodium chloride solution (200 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was dissolved in a mixture of *tert*-butanol (240 mL) and water (240 mL). Potassium carbonate

(49.5 g, 358 mmol, 3.0 equiv), sodium bicarbonate (30.1 g, 358 mmol, 3.0 equiv), potassium ferricyanide (118 g, 358 mmol, 3.0 equiv), and $(DHQ)_2AQN$ (1.02 g, 1.19 mmol, 0.01 equiv) were added in sequence, and the mixture was cooled to 4 °C. Potassium osmate dihydrate (440 mg, 1.19 mmol, 0.01 equiv) was added. After 20 min, methanesulfonamide (11.4 g, 119 mmol, 1.0 equiv) was added. After 10 min, the cooling bath was removed and the reaction flask was allowed to warm to 23 °C. After 25 h, the reaction flask was cooled in an ice bath. Sodium sulfite (150 g, 1.19 mol, 10 equiv) was added slowly. After 30 min, the product mixture was extracted with ethyl acetate (6 × 500 mL). The organic layers were combined. The combined solution was washed sequentially with 1.0 M aqueous sodium hydroxide solution (1.5 L), 1.0 M hydrochloric acid (1.0 L), then saturated aqueous sodium chloride solution (1.0 L) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was recrystallized from dichloromethane–hexanes as in Method A to provide the product, (2*R*,3*S*)-biphenyl-4-ylmethyl 2,3-dihydroxybutanoate (**32**), as light-yellow needles (20.6 g, 60% based on *p*-phenylbenzyl alcohol). The recrystallized product was determined to be of >95% ee by formation of the corresponding bis-Mosher ester as in Method A.

| | |
|---|---|
| **$^1$H NMR**: | 7.60 (t, 4H, $J$ = 8.3 Hz), 7.46 (m, 4H), 7.38 (m, 1H), |
| (500 MHz, $CDCl_3$) | 5.33–5.28 (m, 2H), 4.17 (br, 1H), 4.11 (br, 1H), 3.46 (br, 1H), 2.57 (br, 1H), 1.33 (d, 3H, $J$ = 6.4 Hz). |
| **$^{13}$C NMR**: | 173.2, 141.5, 140.4, 133.9, 128.8, 128.8, 127.5, 127.3, |

| | | |
|---|---|---|
| (125 MHz, $CDCl_3$) | 127, 74.5, 68.7, 67.3, 19.6. | |
| **FTIR**, $cm^{-1}$: | 3335 (br), 1701 (s), 1292 (s), 1138 (m), 1067 (m), 1018 | |
| (neat) | (m), 978 (m), 827 (m). | |
| **HRMS**: | Calcd for $(C_{17}H_{18}O_4+Na)^+$ | 309.1097 |
| (ESI) | Found | 309.1100 |
| **TLC**: | $R_f$ = 0.11 (CAM) | |
| (30% ethyl acetate–hexanes) | | |
| $\mathbf{[\alpha]^{23}_D}$: | +22.7° (*c* 1.04, $CH_2Cl_2$) | |
| **mp**: | 100.0–101.0 °C | |




32 33

(4*R*,5*S*)-Biphenyl-4-ylmethyl 2,2,5-Trimethyl-1,3-dioxolane-4-carboxylate (**33**).

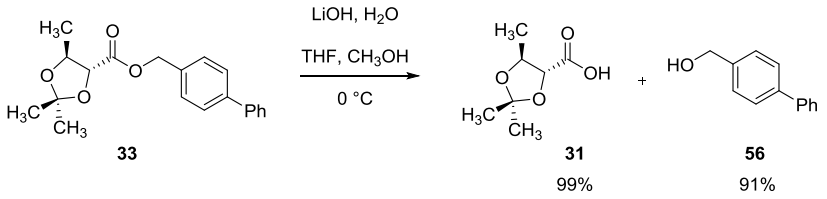
*p*-Toluenesulfonic acid monohydrate (923 mg, 4.85 mmol, 0.05 equiv) was added to a suspension of (2*R*,3*S*)-biphenyl-4-ylmethyl 2,3-dihydroxybutanoate **32** (27.8 g, 97.1 mmol, 1 equiv) in 2,2-dimethoxypropane (350 mL) at 23 °C. After 40 min, triethylamine (677 μL, 4.85 mmol, 0.05 equiv) was added. The product solution was filtered through a pad of silica gel (length: 8 cm; diameter: 8 cm), eluting with ethyl acetate (2 L). The filtrate was concentrated to provide the product, (4*R*,5*S*)-biphenyl-4-ylmethyl 2,2,5-trimethyl-1,3-dioxolane-4-carboxylate (**33**), as a colorless oil (31.4 g, 99%).

| | |
|---|---|
| **$^1$H NMR**: | 7.59 (m, 4H), 7.44 (m, 4H), 7.36 (m, 1H), 5.26 (m, 2H), |
| (500 MHz, $CDCl_3$) | 4.22 (dq, 1H, $J$ = 8.2, 6.0 Hz), 4.11 (d, 1H, $J$ = 7.8 Hz), |
| | 1.48 (s, 3H), 1.45 (s, 3H), 1.44 (d, 3H, $J$ = 6.0 Hz). |
| **$^{13}$C NMR**: | 170.3, 141.3, 140.4, 134.2, 128.7, 128.7, 127.4, 127.3, |
| (125 MHz, $CDCl_3$) | 127, 110.6, 80.3, 75.1, 66.6, 27.1, 25.6, 18.5. |
| **FTIR**, $cm^{-1}$: | 2988 (w), 1757 (s), 1184 (s), 1099 (s), 850 (s), 762 (s), |
| (neat) | 698 (s). |

| | | |
|---|---|---|
| **HRMS**: | Calcd for $(C_{20}H_{22}O_4+Na)^+$ | 349.1410 |
| (ESI) | Found | 349.1416 |

**TLC**: $R_f = 0.67$ (CAM)

(30% ethyl acetate–hexanes)

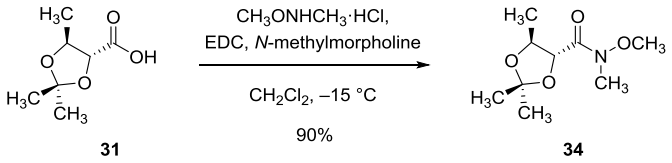



(4*R*,5*S*)-2,2,5-Trimethyl-1,3-dioxolane-4-carboxylic Acid (**31**).

Lithium hydroxide (1.0 M in water, 192 mL, 192 mmol, 2.0 equiv) was added over 5 min to an ice-cooled solution of (4*R*,5*S*)-biphenyl-4-ylmethyl 2,2,5-trimethyl-1,3-dioxolane-4-carboxylate **33** (31.4 g, 96.0 mmol, 1 equiv) in a mixture of tetrahydrofuran (380 mL) and methanol (190 mL). After 10 min, the product mixture was concentrated to remove organic solvents. The aqueous residue was diluted with ether (1 L). The layers were separated. The aqueous layer was extracted with ether (500 mL). The organic layers were combined. The combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide *p*-phenylbenzyl alcohol (**56**) as a white solid (16.1 g, 91%). The aqueous layer was acidified to pH ~2 by addition of 1.0 M aqueous hydrochloric acid solution (~100 mL). The acidified solution was extracted with ethyl acetate (500 mL). The pH of the aqueous layer was observed to increase to ~4 after extraction of the acidic product. The aqueous layer was again acidified to pH ~2 by addition of 1.0 M aqueous hydrochloric acid solution (~50 mL). The acidified solution was extracted with ethyl acetate (3 × 500 mL). The organic layers were combined. The combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the product, (4*R*,5*S*)-2,2,5-trimethyl-1,3-dioxolane-4-carboxylic acid (**31**), as a colorless oil (15.2 g, 99%). $^{1}$H and $^{13}$C NMR data were in agreement with previously reported values.[54]

[54] Klein, U.; Mohrs, K.; Wild, H.; Steglich, W. *Liebigs Ann. Chem.* **1987**, *1987*, 485–489.

| | | |
|---|---|---|
| **$^{1}$H NMR**: (500 MHz, $CDCl_3$) | 10.23 (br, 1H), 4.24 (dq, 1H, $J$ = 8.3, 5.9 Hz), 4.09 (d, 1H, $J$ = 8.3 Hz), 1.49 (s, 3H), 1.48 (d, 3H, $J$ = 5.9 Hz), 1.46 (s, 3H). | |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 175.0, 111.0, 79.8, 75.2, 27.1, 25.6, 18.5. | |
| **FTIR**, $cm^{-1}$: (neat) | 3158 (br), 2990 (w), 1734 (s), 1383 (m), 1219 (s), 1173 (m), 1099 (s), 853 (m). | |
| **HRMS**: (ESI) | Calcd for $(C_7H_{12}O_4+H)^+$ | 161.0808 |
| | Found | 161.0816 |
| **TLC**: (ethyl acetate) | $R_f$ = 0.13 (CAM) | |
| **$[\alpha]^{23}_{D}$**: | +34.2° (*c* 0.61, $CHCl_3$), lit.[54] +31.2° (*c* 1, $CHCl_3$). | |




(4*R*,5*S*)-*N*-Methoxy-*N*,2,2,5-tetramethyl-1,3-dioxolane-4-carboxamide (**34**).

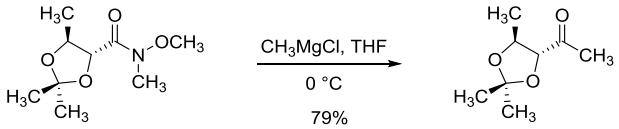
*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (20.1 g, 105 mmol, 1.1 equiv) was added over 2 min to a solution of (4*R*,5*S*)-2,2,5-trimethyl-1,3-dioxolane-4-carboxylic acid **31** (15.2 g, 95.2 mmol, 1 equiv) and *N*-methylmorpholine (11.5 mL, 105 mmol, 1.1 equiv) in dichloromethane (475 mL) at –20 °C. After 3.5 h, 1.0 M aqueous hydrochloric acid solution (150 mL) was added. The cooling bath was removed and the reaction flask was allowed to warm to 23 °C. The layers were separated. The aqueous layer was extracted with dichloromethane (400 mL). The organic layers were combined. The combined solution was washed with saturated aqueous sodium bicarbonate solution (150 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide of the product, (4*R*,5*S*)-*N*-methoxy-*N*,2,2,5-tetramethyl-1,3-dioxolane-4-carboxamide (**34**), as a pale yellow oil (17.4 g, 90%).[55]

| | |
|---|---|
| **$^1$H NMR**: | 4.40 (m, 2H), 3.73 (s, 3H), 3.22 (s, 3H), 1.47 (s, 3H), |
| (500 MHz, $CDCl_3$) | 1.46 (s, 3H), 1.37 (d, 3H, $J$ = 5.9 Hz). |
| **$^{13}$C NMR**: | 170.0, 109.9, 78.2, 74.5, 61.3, 32.0, 27.2, 25.8, 17.9. |

[55] A preparation of amide ent-**34** is described, but characterization data were not reported: Ziegler, F.; Kim, H. *Tetrahedron Lett.* **1993**, *34*, 7669–7672.

(125 MHz, $CDCl_3$)

| | | |
|---|---|---|
| **FTIR**, $cm^{-1}$: | 2986 (w), 2938 (w), 1670 (s), 1371 (m), 1171 (s), 1055 | |
| (neat) | (m), 854 (m). | |
| **HRMS**: | Calcd for $(C_9H_{17}NO_4+Na)^+$ | 226.1050 |
| (ESI) | Found | 226.1050 |
| **TLC**: | $R_f$ = 0.36 (CAM) | |
| (40% ethyl acetate–hexanes) | | |




**34** **35**

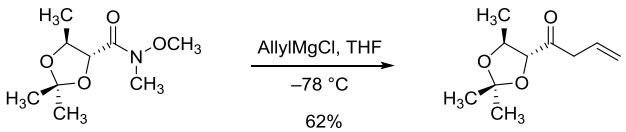
1-((4*R*,5*S*)-2,2,5-Trimethyl-1,3-dioxolan-4-yl)ethanone (**35**).

Methylmagnesium chloride (3.0 M solution in tetrahydrofuran, 6.25 mL, 18.7 mmol, 3.0 equiv) was added dropwise to an ice-cooled solution of (4*R*,5*S*)-*N*-methoxy-*N*,2,2,5-tetramethyl-1,3-dioxolane-4-carboxamide **34** (1.27 g, 6.25 mmol, 1 equiv) in tetrahydrofuran (60 mL). After 35 min, saturated aqueous ammonium chloride solution (30 mL) was added, and the reaction flask was allowed to warm to 23 °C. The product mixture was extracted with ethyl acetate (3 × 70 mL). The organic layers were combined. The combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the product, 1-((4*R*,5*S*)-2,2,5-trimethyl-1,3-dioxolan-4-yl)ethanone (**35**), as a colorless oil (770 mg, 79%). $^{1}$H NMR, $^{13}$C NMR, IR, and MS data were in agreement with previously reported values.[56]

**TLC**: $R_f$ = 0.70 (CAM)

(30% ethyl acetate–hexanes)

[56] For characterization data for ent-**35**, see: Aoyagi, S.; Wang, T. C.; Kibayashi, C. *J. Am. Chem. Soc.* **1993**, *115*, 11393–11409.



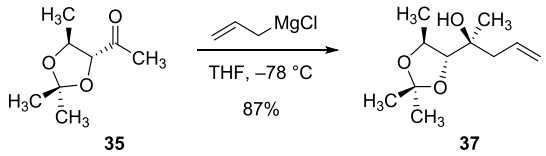

34 36

1-((4*R*,5*S*)-2,2,5-trimethyl-1,3-dioxolan-4-yl)but-3-en-1-one (**36**).

Allylmagnesium chloride (2.0 M solution in tetrahydrofuran, 1.48 mL, 2.95 mmol, 3.0 equiv) was added dropwise to a solution of (4*R*,5*S*)-*N*-methoxy-*N*,2,2,5-tetramethyl-1,3-dioxolane-4-carboxamide **34** (200 mg, 0.984 mmol, 1 equiv) in tetrahydrofuran (10 mL) at –78 °C. After 25 min, saturated aqueous ammonium chloride (5 mL) was added, and the reaction flask was allowed to warm to 23 °C. Water (2 mL) was added and the product mixture was extracted with ethyl acetate (2 × 20 mL). The organic layers were combined. The combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (4% ethyl acetate–hexanes) to provide the product, 1-((4*R*,5*S*)-2,2,5-trimethyl-1,3-dioxolan-4-yl)but-3-en-1-one (**36**), as a colorless oil (113 mg, 62%).

| | |
|---|---|
| **$^1$H NMR**: (500 MHz, $CDCl_3$) | 5.94 (m, 1H), 5.22–5.14 (m, 2H), 4.05 (dq, 1H, $J$ = 8.3, 6.4 Hz), 3.93 (dd, 1H, $J$ = 8.3, 1.0 Hz), 3.43 (m, 2H), 1.46 (s, 3H), 1.44 (s, 3H), 1.39 (d, 3H, $J$ = 5.9 Hz). |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 207.7, 129.7, 119.1, 110.1, 86.3, 74.3, 43.3, 27.2, 26.3, 18.5. |

| | | |
|---|---|---|
| **FTIR**, $cm^{-1}$: | 2987 (m), 1719 (s), 1382 (s), 1242 (s), 1099 (s), 856 (s). | |
| (neat) | | |
| **HRMS**: | Calcd for $(C_{10}H_{16}O_3+H)^+$ | 185.1172 |
| (ESI) | Found | 185.1179 |
| **TLC**: | $R_f$ = 0.75 (CAM) | |
| (30% ethyl acetate–hexanes) | | |




35 37

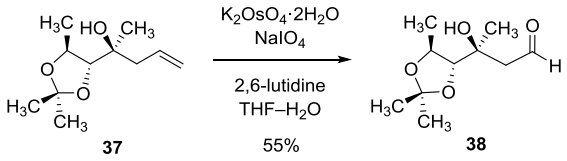
(*S*)-2-((4*R*,5*S*)-2,2,5-Trimethyl-1,3-dioxolan-4-yl)pent-4-en-2-ol (**37**).

Allylmagnesium chloride (2.0 M solution in tetrahydrofuran, 2.92 mL, 5.84 mmol, 2.2 equiv) was added dropwise to a solution of 1-((4*R*,5*S*)-2,2,5-trimethyl-1,3-dioxolan-4-yl)ethanone **35** (420 mg, 2.66 mmol, 1 equiv) in tetrahydrofuran (25 mL) at –78 °C. After 10 min, saturated aqueous ammonium chloride solution (5 mL) was added carefully. The cooling bath was removed and the reaction flask was allowed to warm to 23 °C. The mixture was extracted with dichloromethane (3 × 30 mL). The organic layers were combined. The combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide (*S*)-2-((4*R*,5*S*)-2,2,5-trimethyl-1,3-dioxolan-4-yl)pent-4-en-2-ol (**37**) as a colorless oil (460 mg, 87%).[57]

| | |
|---|---|
| **$^1$H NMR**: (500 MHz, $CDCl_3$) | 5.94–5.86 (m, 1H), 5.19–5.13 (m, 2H), 4.15–4.10 (m, 1H), 3.50 (d, 1H, *J* = 7.8 Hz), 2.35 (dd, 1H, *J* = 13.7, 6.8 Hz), 2.15 (dd, 1H, *J* = 13.7, 7.8 Hz), 1.97 (s, 1H), 1.41 (s, 3H), 1.40 (s, 3H), 1.35 (d, 3H, *J* = 5.9 Hz), 1.21 (s, 3H). |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 133.0, 118.7, 107.7, 87.4, 72.5, 71.3, 42.6, 27.3, 26.8, 23.5, 20.0 |

[57] The product obtained in this procedure is a 4:1 mixture of diastereomers (epimeric tertiary alcohols); the major diastereomer is depicted in the equation above. The minor epimer was not easily separable by chromatography and was carried through as an impurity.

| | | |
|---|---|---|
| **FTIR**, $cm^{-1}$: | 3481 (br), 2984 (m), 1378 (s), 122 (s), 1174 (s), 1082 (s), | |
| (neat) | 1055 (s), 916 (m). | |
| **HRMS**: | Calcd for $(C_{11}H_{20}O_3+Na)^+$ | 223.1305 |
| (ESI) | Found | 223.1280 |
| **TLC**: | $R_f$ = 0.59 (CAM) | |
| (30% ethyl acetate–hexanes) | | |

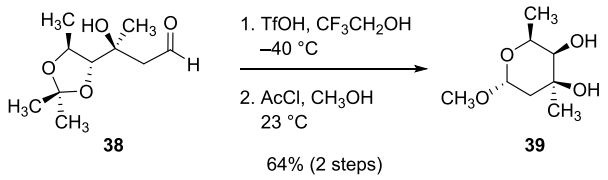



(*S*)-3-Hydroxy-3-((4*R*,5*S*)-2,2,5-trimethyl-1,3-dioxolan-4-yl)butanal (**38**).

2,6-Lutidine (341 µL, 2.95 mmol, 2.0 equiv) was added to a vigorously stirring suspension of (*S*)-2-((4*R*,5*S*)-2,2,5-trimethyl-1,3-dioxolan-4-yl)pent-4-en-2-ol **37** (295 mg, 1.47 mmol, 1 equiv), potassium osmate dihydrate (27 mg, 0.074 mmol, 0.05 equiv), and sodium periodate (1.26 g, 5.89 mmol, 4.0 equiv) in a mixture of tetrahydrofuran (20 mL) and water (10 mL). After 4 h, saturated aqueous sodium chloride solution was added (50 mL). The mixture was extracted with 1:1 ethyl acetate–hexanes (3 × 60 mL). The organic layers were combined. The combined solution was washed sequentially with saturated aqueous copper(II) sulfate solution (50 mL) then saturated aqueous sodium chloride solution (50 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (10% ethyl acetate–hexanes initially, grading to 30% ethyl acetate–hexanes) to provide (*S*)-3-hydroxy-3-((4*R*,5*S*)-2,2,5-trimethyl-1,3-dioxolan-4-yl)butanal (**38**) as a pale brown oil (166 mg, 56%).

| | |
|---|---|
| **$^1$H NMR**: | 9.88 (m, 1H), 4.02 (m, 1H), 3.57 (d, 1H, $J$ = 8.2 Hz), |
| (500 MHz, $CDCl_3$) | 2.88 (br, 1H), 2.75 (d, 1H, $J$ = 16.0 Hz), 2.46 (dt, 1H, $J$ = 16.5, 1.8 Hz), 1.38 (s, 3H), 1.37 (s, 3H), 1.32 (d, 3H, $J$ = 6.0 Hz), 1.31 (s, 3H). |

| | | |
|---|---|---|
| **$^{13}$C NMR**: | 202.3, 108.4, 87.1, 72.8, 71.6, 50.8, 27.3, 26.9, 24.1, | |
| (125 MHz, $CDCl_3$) | 20.0. | |
| **FTIR**, $cm^{-1}$: | 3475 (br) 2985 (m), 1718 (s), 1379 (s), 1217 (s), 1173 | |
| (neat) | (s), 1077 (s), 862 (m). | |
| **HRMS**: | Calcd for $(C_{10}H_{18}O_4+Na)^+$ | 225.1113 |
| (ESI) | Found | 225.1097 |
| **TLC**: | $R_f$ = 0.26 (CAM) | |
| (30% ethyl acetate–hexanes) | | |




**38** **39**

Confirmation of Stereochemistry: Methyl 2,6-Dideoxy-3-*C*-methyl-L-*lyxo*-hexopyranoside (Methyl 3-*epi*-Axenoside, **39**).

Triflic acid (5% solution in 2,2,2-trifluoroethanol, 56 μL, 0.05 equiv) was added to a solution of (*S*)-3-hydroxy-3-((4*R*,5*S*)-2,2,5-trimethyl-1,3-dioxolan-4-yl)butanal **38** (128 mg, 0.633 mmol, 1 equiv) in 2,2,2-trifluoroethanol (9.9 mL) at –40 °C. After 40 min, triethylamine (10 μL) was added. The cooling bath was removed and the product solution was concentrated. $^1$H NMR analysis of the residue established that the acetonide protective group had been removed. The residue was dissolved in methanol (6.3 mL). Acetyl chloride (451 μL, 6.35 mmol, 10 equiv) was added dropwise at 23 °C. After 20 min, silver carbonate (2.1 g, 7.62 mmol, 12 equiv) was added. After 10 min, the product mixture was filtered through a pad of Celite. The filtrate was concentrated. The residue was purified by flash-column chromatography (50% ethyl acetate–hexanes) to provide the product (72 mg, 64% yield over two steps) as a colorless oil. $^1$H NMR analysis revealed that the purified product was a mixture of α and β methyl glycosides. The most abundant component of the mixture exhibited $^1$H NMR data in full agreement with those reported for methyl α-3-*epi*-axenoside (**39**).[58]

**$^1$H NMR**: (500 MHz, $CDCl_3$) 4.74 (d, 1H, $J$ = 4.4 Hz), 3.98 (q, 1H, $J$ = 6.4 Hz), 3.31 (s, 3H), 3.19 (s, 1H), 2.67 (br, 1H), 2.28 (br, 1H), 1.75–1.87

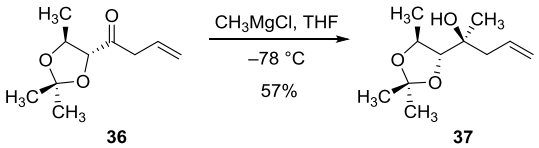
[58] Roush, W. R.; Hagadorn, S. M. *Carbohyd. Res.* **1985**, *136*, 187–193.

(m, 2H), 1.39 (s, 3H), 1.28 (d, 3H, $J$ = 6.4 Hz).

Methyl β-axenoside was observed as a minor impurity:[10]

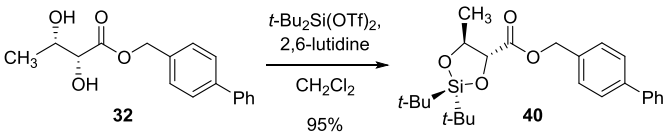
**$^1$H NMR**: 4.61 (dd, 1H, $J$ = 9.3, 2.4 Hz), 4.12 (q, 1H, $J$ = 6.4 Hz).

(500 MHz, $CDCl_3$)




(*S*)-2-((4*R*,5*S*)-2,2,5-Trimethyl-1,3-dioxolan-4-yl)pent-4-en-2-ol (**37**).

Methylmagnesium chloride (3.0 M solution in tetrahydrofuran, 288 μL, 0.863 mmol, 1.5 equiv) was added dropwise to a solution of 1-((4*R*,5*S*)-2,2,5-trimethyl-1,3-dioxolan-4-yl)but-3-en-1-one **36** (106 mg, 0.575 mmol, 1 equiv) in tetrahydrofuran (6.0 mL) at –78 °C. After 45 min, a second portion of methylmagnesium chloride (3.0 M solution in tetrahydrofuran, 95 μL, 0.288 mmol, 0.5 equiv) was added. After 15 min, saturated aqueous ammonium chloride solution (2 mL) was added carefully. The cooling bath was removed and the reaction flask was allowed to warm to 23 °C. The mixture was extracted with dichloromethane (3 × 10 mL). The organic layers were combined. The combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (10% ethyl acetate–hexanes) to provide (*S*)-2-((4*R*,5*S*)-2,2,5-trimethyl-1,3-dioxolan-4-yl)pent-4-en-2-ol (**37**) as a colorless oil (65 mg, 57%). The product obtained in this procedure is a 4:1 mixture of diastereomers (epimeric tertiary alcohols); the major diastereomer is depicted in the equation above. See above for characterization data.

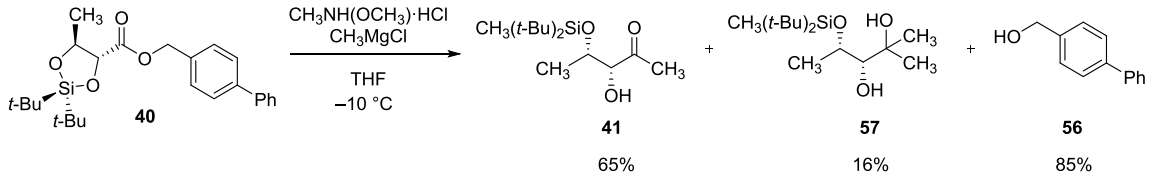



(4*R*,5*S*)-Biphenyl-4-ylmethyl 2,2-Di-*tert*-butyl-5-methyl-1,3,2-dioxasilolane-4-carboxylate (**40**).

Di-*tert*-butylsilyl bis(trifluoromethanesulfonate) (12.6 mL, 34.6 mmol, 1.1 equiv) was added to a suspension of 2,6-lutidine (11.0 mL, 94.0 mmol, 3.0 equiv) and (2*R*,3*S*)-biphenyl-4-ylmethyl 2,3-dihydroxybutanoate **32** (9.00 g, 31.4 mmol, 1 equiv) in dichloromethane (80 mL) at 23 °C. After 30 h, benzene (100 mL) was added and the solution was filtered through a column of silica gel, eluting with 10% ethyl acetate–hexanes. The filtrate was concentrated to provide (4*R*,5*S*)-biphenyl-4-ylmethyl 2,2-di-*tert*-butyl-5-methyl-1,3,2-dioxasilolane-4-carboxylate (**40**) as a colorless oil (12.71 g, 95%).

| | |
|---|---|
| **$^1$H NMR**: (500 MHz, $CDCl_3$) | 7.59 (d, 4H, *J* = 7.8 Hz), 7.46–7.42 (m, 4H), 7.36 (t, 1H, *J* = 7.3 Hz), 5.31 (d, 1H, *J* = 12.2 Hz), 5.19 (d, 1H, *J* = 12.2 Hz), 4.18–4.10 (m, 2H), 1.44 (d, 3H, *J* = 5.9 Hz), 1.08 (s, 9H), 1.05 (s, 9H). |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 170.9, 141.2, 140.4, 134.3, 128.7, 128.6, 127.4, 127.2, 127.0, 80.2, 74.4, 66.4, 26.7, 26.7, 21.3, 21.0, 20.5. |
| **FTIR**, $cm^{-1}$: | 2934 (m), 1759 (m), 1473 (m), 1279 (m), 1119 (s), 1090 |

| | | |
|---|---|---|
| (neat) | (s), 928 (m), 826 (s). | |
| **HRMS**: | Calcd for $(C_{25}H_{34}O_4Si+Na)^+$ | 449.2119 |
| (ESI) | Found | 449.2122 |
| **TLC**: | $R_f$ = 0.82 (CAM) | |
| (30% ethyl acetate–hexanes) | | |




(3*R*,4*S*)-4-(Di-*tert*-butyl(methyl)silyloxy)-3-hydroxypentan-2-one (**41**).

Methylmagnesium chloride (3.0 M solution in tetrahydrofuran, 79.0 mL, 238 mmol, 8.0 equiv) was added dropwise through an addition funnel over 20 min to a suspension of *N*,*O*-dimethylhydroxylamine hydrochloride (3.63 g, 37.2 mmol, 1.25 equiv) and (4*R*,5*S*)-biphenyl-4-ylmethyl 2,2-di-*tert*-butyl-5-methyl-1,3,2-dioxasilolane-4-carboxylate **40** (12.71 g, 29.8 mmol, 1 equiv) in tetrahydrofuran (300 mL) at –10 °C. After 10 min, saturated aqueous ammonium chloride solution (50 mL) was added carefully. The cooling bath was removed and the reaction flask was allowed to warm to 23 °C. Water (200 mL) was added, and the mixture was extracted with ether (4 × 200 mL). The organic layers were combined. The combined solution was washed with saturated aqueous sodium chloride solution (100 mL) and the washed solution was dried over magnesium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (5% ethyl acetate–hexanes initially, grading to 30% ethyl acetate–hexanes) to provide (3*R*,4*S*)-4-(Di-*tert*-butyl(methyl)silyloxy)-3-hydroxypentan-2-one (**41**) as a colorless oil (5.34 g, 65%). In addition to the desired product, the tertiary alcohol byproduct, (3*R*,4*S*)-4-(di-*tert*-butyl(methyl)silyloxy)-2-methylpentane-2,3-diol (**57**), was obtained as a colorless oil (1.36 g, 16%). Finally, a fraction containing *p*-phenylbenzyl alcohol (**56**) (~90% purity) was collected. Recrystallization from dichloromethane–hexanes furnished pure *p*-phenylbenzyl alcohol as a white solid (≥95% purity, 4.68 g, 85%).

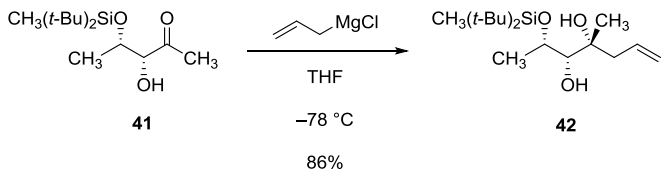
(3*R*,4*S*)-4-(Di-*tert*-butyl(methyl)silyloxy)-3-hydroxypentan-2-one (**41**):

| | | |
|---|---|---|
| **$^1$H NMR**: | 4.19 (m, 1H), 4.14 (m, 1H), 3.62 (d, 1H, $J$ = 5.5 Hz), | |
| (500 MHz, $CDCl_3$) | 2.31 (s, 3H), 1.05 (d, 1H, $J$ = 6.4 Hz), 0.98 (s, 9H), 0.95 (s, 9H), 0.09 (s, 3H). | |
| **$^{13}$C NMR**: | 209.6, 80.4, 70.5, 27.8, 27.7, 27.6, 20.9, 20.1, 18.4, –8.9. | |
| (125 MHz, $CDCl_3$) | | |
| **FTIR**, $cm^{-1}$: | 3482 (br), 2934 (m), 1715 (m), 1472 (m), 1256 (m), 1105 | |
| (neat) | (s), 1007 (m), 824 (s). | |
| **HRMS**: | Calcd for $(C_{14}H_{30}O_3Si+H)^+$ | 275.2037 |
| (ESI) | Found | 275.2034 |
| **TLC**: | $R_f$ = 0.60 (CAM) | |
| (20% ethyl acetate–hexanes) | | |

(3*R*,4*S*)-4-(Di-*tert*-butyl(methyl)silyloxy)-2-methylpentane-2,3-diol (**57**):

| | |
|---|---|
| **$^1$H NMR**: | 4.30–4.25 (m, 1H), 3.08–3.04 (m, 2H), 2.83 (s, 1H), 1.31 |
| (500 MHz, $CDCl_3$) | (d, 3H, $J$ = 6.4 Hz), 1.26 (s, 6H), 1.02 (s, 9H), 0.99 (s, 9H). |

| | | |
|---|---|---|
| **$^{13}$C NMR**: | 80.1, 72.3, 69.8, 28.0, 27.8, 26.8, 26.1, 22.7, 21.2, 20.2, | |
| (125 MHz, $CDCl_3$) | –6.8. | |
| **FTIR**, $cm^{-1}$: | 3470 (br), 2859 (m), 1471 (m), 1256 (m), 1128 (m), 1060 | |
| (neat) | (s), 934 (s), 823 (s). | |
| **HRMS**: | Calcd for $(C_{15}H_{34}O_3Si+H)^+$ | 291.2350 |
| (ESI) | Found | 291.2362 |
| **TLC**: | $R_f$ = 0.38 (CAM) | |
| (20% ethyl acetate–hexanes) | | |

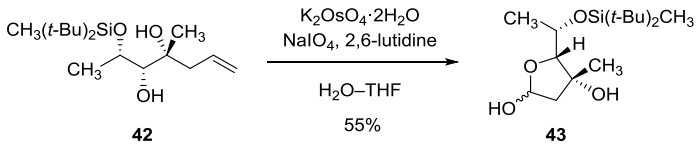



(2*S*,3*R*,4*R*)-2-(Di-*tert*-butyl(methyl)silyloxy)-4-methylhept-6-ene-3,4-diol (**42**).

Allylmagnesium chloride (2.0 M solution in tetrahydrofuran, 19.8 mL, 39.6 mmol, 3.0 equiv) was added dropwise to a solution of (3*R*,4*S*)-4-(di-*tert*-butyl(methyl)silyloxy)-3-hydroxypentan-2-one **41** (3.62 g, 13.2 mmol, 1 equiv) in tetrahydrofuran (88 mL) at –78 °C. After 3 h, a second portion of allylmagnesium chloride (2.0 M solution in tetrahydrofuran, 6.6 mL, 13.2 mmol, 1.0 equiv) was added. After 1 h, the cooling bath was removed. After 10 min, aqueous ammonium chloride solution (50% saturated, 25 mL) was added slowly, and the reaction flask was allowed to warm to 23 °C. Aqueous sodium chloride solution (25% saturated, 50 mL), and methyl *tert*-butyl ether (50 mL) were added. The layers were separated. The aqueous layer was extracted with methyl *tert*-butyl ether (2 × 100 mL). The organic layers were combined. The combined solution was washed with saturated aqueous sodium chloride solution (50 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (5% ethyl acetate–hexanes) to provide (2*S*,3*R*,4*R*)-2-(di-*tert*-butyl(methyl)silyloxy)-4-methylhept-6-ene-3,4-diol (**42**) as a colorless oil (3.59 g, 86%).[59]

[59] The product obtained in this procedure is a 13:1 mixture of diastereomers (epimeric tertiary alcohols); the major diastereomer is depicted in the equation above. The minor epimer was carried through as an impurity to the stage of the oxidative cleavage reaction (vide infra), where the diastereomeric products were easily separated.

| | | |
|---|---|---|
| **$^{1}$H NMR**: (500 MHz, $CDCl_3$) | 5.86 (m, 1H), 5.12 (m, 1H), 5.10 (t, 1H, $J$ = 1.5 Hz), 4.3 (dq, 1H, $J$ = 5.9, 3.9 Hz), 3.11 (dd, 1H, $J$ = 6.8, 3.4 Hz), 3.05 (d, 1H, $J$ = 6.8 Hz), 2.90 (s, 1H), 2.26 (m, 2H), 1.30 (d, 3H, $J$ = 6.4 Hz), 1.20 (s, 3H), 1.02 (s, 9H), 0.99 (s, 9H), 0.17 (s, 3H). | |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 134.0, 118.1, 77.9, 73.9, 69.6, 44.1, 28.0, 27.9, 23.2, 22.8, 21.3, 20.3, –6.7. | |
| **FTIR**, $cm^{-1}$: (neat) | 3503 (br), 2934 (m), 1472 (m), 1256 (m), 1065 (s), 928 (m), 824 (s). | |
| **HRMS**: (ESI) | Calcd for $(C_{17}H_{36}O_3Si+Na)^+$ | 339.2326 |
| | Found | 339.2330 |
| **TLC**: (20% ethyl acetate–hexanes) | $R_f$ = 0.60 (CAM) | |



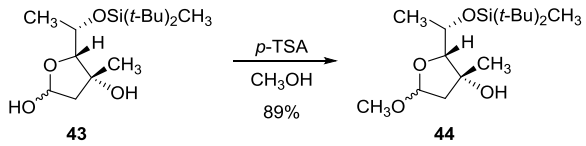

5-*O*-(Di-*tert*-butylmethylsilyl)-2,6-dideoxy-3-*C*-methyl-L-*xylo*-hexofuranose (**43**).

Potassium osmate dihydrate (209 mg, 0.567 mmol, 0.05 equiv) was added to a solution of (2*S*,3*R*,4*R*)-2-(di-*tert*-butyl(methyl)silyloxy)-4-methylhept-6-ene-3,4-diol **42** (3.59 g, 11.3 mmol, 1 equiv), sodium periodate (9.70 g, 45.4 mmol, 4.0 equiv), and 2,6-lutidine (2.64 mL, 22.7 mmol, 2.0 equiv) in a mixture of tetrahydrofuran (76 mL) and water (38 mL) at 23 °C. After 3 h, the product mixture was partitioned between saturated aqueous sodium chloride solution (100 mL), ethyl acetate (50 mL), and hexanes (50 mL). The layers were separated. The aqueous layer was extracted with 50% ethyl acetate–hexanes (2 × 100 mL). The organic layers were combined. The combined solution was washed with saturated aqueous copper(II) sulfate solution (100 mL) then saturated aqueous sodium chloride solution (100 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (10% ethyl acetate–hexanes initially, grading to 20% ethyl acetate–hexanes) to provide 5-*O*-(di-*tert*-butylmethylsilyl)-2,6-dideoxy-3-*C*-methyl-L-*xylo*-hexofuranose (**43**) as a 1:1.9 mixture of α- and β-anomers, respectively (1.97 g, 55%).

**$^1$H NMR**: (500 MHz, $CDCl_3$)

**α-anomer (minor):** 5.66 (m, 1H), 4,35 (m, 1H) 4.17 (s, 1H), 3.79 (d, 1H, *J* = 3.9 Hz), 2.61 (br, 1H), 2.33 (dd, 1H, *J* = 13.7, 5.9 Hz), 1.92 (dd, 1H, *J* = 13.7, 3.4 Hz),

| | |
|---|---|
| | 1.48 (s, 3H), 1.37 (d, 3H, $J$ = 6.4 Hz), 1.01 (s, 9H), 0.98 (s, 9H), 0.16 (s, 3H). |
| | **β-anomer (major):** 5.41 (dd, 1H, $J$ = 8.8, 4.9 Hz), 4.46 (s, 1H), 4.35 (m, 1H), 3.68 (d, 1H, $J$ = 8.8 Hz), 3.48 (d, 1H, $J$ = 4.4 Hz), 2.52 (s, 1H), 2.10 (d, 1H, $J$ = 13.7 Hz), 2.02 (dd, 1H, $J$ = 13.2, 4.9 Hz), 1.42 (s, 3H), 1.38 (d, 3H, $J$ = 6.4 Hz), 1.03 (s, 9H), 1.00 (s, 9H), 0.18 (s, 3H). |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 97.8, 97.5, 89.0, 85.8, 79.2, 78.1, 70.7, 70.1, 50.1, 48.7, 27.9, 27.9, 27.8, 27.7, 27.3, 24.6, 21.2, 21.2, 20.9, 20.5, 20.3, 20.2, –7.1, –7.2. |
| **FTIR**, $cm^{-1}$: (neat) | 3420 (br), 2934 (m), 1472 (m), 1256 (m), 1086 (s), 934 (m), 824 (s). |
| **HRMS**: (ESI) | Calcd for $(C_{16}H_{34}O_4Si+Na)^+$ 341.2119<br>Found 341.2129 |
| **TLC**: (20% ethyl acetate–hexanes) | $R_f$ = 0.27 (CAM) |




Methyl 5-*O*-(Di-*tert*-butylmethylsilyl)-2,6-dideoxy-3-*C*-methyl-L-*xylo*-hexofuranoside (**44**).

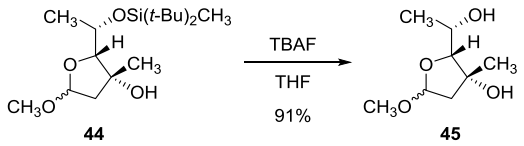
*p*-Toluenesulfonic acid monohydrate (353 mg, 1.86 mmol, 0.3 equiv) was added to a solution of 5-*O*-(di-*tert*-butylmethylsilyl)-2,6-dideoxy-3-*C*-methyl-L-*xylo*-hexofuranose **43** (1.97 g, 6.18 mmol, 1 equiv) in methanol (16 mL) at 23 °C. After 50 min, triethylamine (300 μL) was added and the product solution was concentrated. The residue was filtered through a short pad of silica gel (length: 2.5 cm, diameter: 3.5 cm), eluting with ethyl acetate (60 mL). The filtrate was concentrated to provide methyl 5-*O*-(di-*tert*-butylmethylsilyl)-2,6-dideoxy-3-*C*-methyl-L-*xylo*-hexofuranoside (**44**) as a 1:4.8 mixture of α- and β-anomers, respectively (1.84 g, 89%).

**$^1$H NMR**: (600 MHz, $CDCl_3$)

**α-anomer (minor):** 4.93 (dd, 1H, *J* = 4.4, 0.9 Hz), 4.05 (m, 1H), 3.54 (d, 1H, *J* = 7.0 Hz), 3.45 (s, 1H), 3.37 (s, 3H), 2.07–2.01 (m, 2H), 1.36 (s, 3H), 1.28 (d, 3H, *J* = 6.2 Hz), 1.00 (s, 9H), 0.96 (s, 9H), 0.11 (s, 3H).

**β-anomer (major):** 5.06 (dd, 1H, *J* = 5.9, 2.9 Hz), 4.30 (dq, 1H, *J* = 6.4, 4.4 Hz), 3.80 (s, 1H), 3.64 (d, 1H, *J* = 4.1 Hz), 3.33 (s, 3H), 2.26 (dd, 1H, *J* = 13.7, 5.9 Hz), 1.92 (dd, 1H, *J* = 13.8, 2.6 Hz), 1.43 (s, 3H), 1.34 (d, 3H,

| | | |
|---|---|---|
| | $J$ = 6.4 Hz), 1.00 (s, 9H), 0.96 (s, 9H), 0.13 (s, 3H). | |
| **$^{13}$C NMR**: (100 MHz, $CDCl_3$) | 103.9, 103.7, 91.8, 85.6, 78.9, 77.0, 71.8, 70.3, 55.4, 55.1, 49.7, 48.5, 27.9, 27.9, 27.8, 27.7, 24.5, 21.5, 21.1, 21.0, 20.5, 20.3, 20.3, 20.2, –7.5, –8.1. | |
| **FTIR**, $cm^{-1}$: (neat) | 3482 (br), 2934 (m), 1472 (m), 1254 (m), 1105 (s), 1044 (s). | |
| **HRMS**: (ESI) | Calcd for $(C_{17}H_{36}O_4Si+Na)^+$ | 355.2275 |
| | Found | 355.2266 |
| **TLC**: (20% ethyl acetate–hexanes) | $R_f$ = 0.49, 0.69 (CAM) | |




Methyl 2,6-Dideoxy-3-*C*-methyl-L-*xylo*-hexofuranoside (**45**).

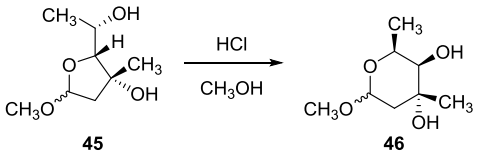
Tetra-*n*-butylammonium fluoride (1.0 M solution in tetrahydrofuran, 6.07 mL, 6.07 mmol, 1.1 equiv) was added to a solution of methyl 5-*O*-(di-*tert*-butylmethylsilyl)-2,6-dideoxy-3-*C*-methyl-L-*xylo*-hexofuranoside **44** (1.836 g, 5.52 mmol, 1 equiv) in tetrahydrofuran (37 mL) at 23 °C. After 16 h, the reaction mixture was partitioned between saturated aqueous sodium bicarbonate solution (10 mL) and ethyl acetate (50 mL). The layers were separated. The aqueous layer was extracted with ethyl acetate (3 × 50 mL). The organic layers were combined. The combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (10% ethyl acetate–hexanes initially, grading to 30% ethyl acetate–hexanes, then 80% ethyl acetate–hexanes, then ethyl acetate) to provide methyl 2,6-dideoxy-3-*C*-methyl-L-*xylo*-hexofuranoside (**45**) as a 1:5.0 mixture of α- and β-anomers, respectively (890 mg, 91%).

**$^1$H NMR**: (500 MHz, $CDCl_3$)

**α-anomer (minor):** 5.02 (d, 1H, *J* = 4.9 Hz), 4.01 (m, 1H), 3.66 (s, 1H), 3.55 (s, 1H), 3.42 (s, 3H), 3.02 (d, 1H, *J* = 5.0 Hz), 2.17–2.08 (m, 2H), 1.38 (s, 3H), 1.28 (d, 3H, *J* = 6.4).

**β-anomer (major):** 5.16 (dd, 1H, *J* = 5.9, 3.4 Hz), 4.11

| | | |
|---|---|---|
| | (m, 1H), 3.73 (s, 1H), 3.56 (d, 1H, $J$ = 1.5 Hz), 3.39 (s, 3H), 2.44 (d, 1H, $J$ = 7.9 Hz), 2.30 (dd, 1H, $J$ = 13.8, 5.6 Hz), 1.94 (dd, 1H, $J$ = 13.8, 3.2 Hz), 1.39 (s, 3H), 1.36 (d, 3H, $J$ = 6.7 Hz). | |
| **$^{13}$C NMR**: (100 MHz, $CDCl_3$) | 104.1, 103.8, 90.2, 85.0, 79.1, 77.6, 67.2, 65.9, 55.3, 55.0, 49.6, 48.1, 25.4, 25.2, 21.4, 19.6. | |
| **FTIR**, $cm^{-1}$: (neat) | 3385 (br), 1375 (m), 1285 (w), 1198 (m), 1101 (s), 1028 (s), 970 (m). | |
| **HRMS**: (ESI) | Calcd for $(C_8H_{16}O_4+Na)^+$ | 199.0941 |
| | Found | 199.0932 |
| **TLC**: (80% ethyl acetate–hexanes) | $R_f$ = 0.38 (CAM) | |




45 46

Methyl 2,6-Dideoxy-3-*C*-methyl-L-*xylo*-hexopyranoside (Methyl Axenoside, **46**).

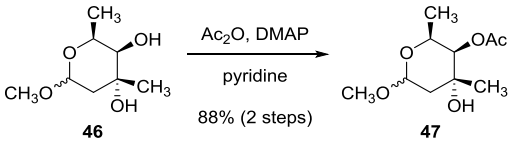
Hydrochloric acid (1.0 M solution in methanol, 5.60 mL, 5.60 mmol, 1.1 equiv) was added to a solution of methyl 2,6-dideoxy-3-C-methyl-L-*xylo*-hexofuranoside **45** (890 mg, 5.05 mmol, 1 equiv) in methanol (45 mL) at 23 °C. After 22 h, silver carbonate (2.23 g, 8.08 mmol, 1.6 equiv) was added. After 30 min, the product mixture was filtered through a short pad of Celite, washing with methanol (50 mL). The filtrate was concentrated to provide the product, methyl 2,6-dideoxy-3-*C*-methyl-L-*xylo*-hexopyranoside (**46**), as a 1:1.8 mixture of α- and β-anomers, respectively, which was used directly in the next reaction without purification.[48]

**$^1$H NMR**: (500 MHz, $CDCl_3$)

**α-anomer (minor):** 4.80 (d, 1H, *J* = 3.9 Hz), 4.30 (q, 1H, *J* = 6.8 Hz), 4.02 (s, 1H), 3.38 (s, 3H), 3.14 (d, 1H, *J* = 7.8 Hz), 1.91 (dd, 1H, *J* = 14.7, 3.9 Hz), 1.73 (d, 1H, *J* = 8.3 Hz), 1.26 (d, 3H, *J* = 6.8 Hz), 1.24 (s, 3H).

**β-anomer (major):** 4.62 (dd, 1H, *J* = 9.8, 2.4 Hz), 4.13 (dq, 1H, *J* = 6.8, 1.0 Hz), 3.50 (s, 3H), 2.98 (d, 1H, *J* = 10.3 Hz), 1.97 (d, 1H, *J* = 9.8 Hz), 1.69–1.57 (m, 2H), 1.34 (s, 3H), 1.27 (d, 3H, *J* = 6.8 Hz).

| | | |
|---|---|---|
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 100.4, 99.0, 74.6, 74.4, 72.5, 70.2, 68.9, 62.5, 56.5, 55.2, 39.1, 35.4, 27.8, 26.0, 16.7, 16.6. | |
| **FTIR**, $cm^{-1}$: (neat) | 3447 (br), 2934 (m), 1375 (m), 1121 (s), 1059 (s), 995 (s). | |
| **HRMS**: (ESI) | Calcd for $(C_8H_{16}O_4+Na)^+$ | 199.0941 |
| | Found | 199.0929 |
| **TLC**: (5% methanol–dichloromethane) | $R_f$ = 0.26, 0.36 (CAM) | |




Methyl Trioxacarcinoside A (**47**).

Acetic anhydride (500 μL, 5.30 mmol, 1.05 equiv) was added to a solution of methyl 2,6-dideoxy-3-*C*-methyl-L-*xylo*-hexopyranoside **46** (1 equiv, see paragraph above) and 4-dimethylaminopyridine (1.23 g, 10.1 mmol, 2.0 equiv) in pyridine (50 mL) at 23 °C. After 20 h, a second portion of acetic anhydride (100 μL, 1.06 mmol, 0.2 equiv) was added. After 90 min, a third portion of acetic anhydride (150 μL, 1.59 mmol, 0.3 equiv) was added. After 40 min, the product solution was partitioned between ethyl acetate (150 mL) and saturated aqueous sodium bicarbonate solution (20 mL). The layers were separated. The organic layer was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (20% ethyl acetate–hexanes initially, grading to 60% ethyl acetate–hexanes) to provide methyl trioxacarcinoside A (**47**) as a 1:1.7 mixture of α- and β-anomers, respectively (970 mg, 88% yield over two steps). For characterization purposes, a sample of the product (~50 mg) was purified by flash-column chromatography (20% ethyl acetate–hexanes initially, grading to 60% ethyl acetate–hexanes) to provide separately α- and β-methyl trioxacarcinoside A (**47**), which were characterized by $^{1}$H NMR, $^{13}$C NMR, IR, and HRMS. Analytical data for the synthetic substances were in agreement with those reported in the literature.[6]

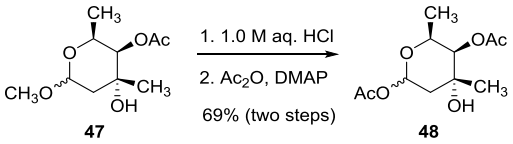
α-anomer (minor):

| | | |
|---|---|---|
| **$^{1}$H NMR**: (500 MHz, $CDCl_3$) | 4.86 (d, 1H, *J* = 3.4 Hz), 4.70 (s, 1H), 4.32 (q, 1H, *J* = 6.4 Hz), 4.28 (s, 1H), 3.39 (s, 3H), 2.14 (s 3H), 1.93 (dd, 1H, *J* = 14.2, 3.9 Hz), 1.72 (dt, 1H, *J* = 14.7, 1.2 Hz), 1.13 (d, 3H, *J* = 6.4 Hz), 1.10 (s, 3H). | |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 170.4, 99.1, 74.6, 68.8, 61.8, 55.2, 36.2, 25.7, 20.9, 16.6. | |
| **FTIR**, $cm^{-1}$: (neat) | 3507 (br, w), 2938 (w), 1748 (s), 1375 (m), 1233 (s), 1140 (m), 1121 (s), 1082 (m), 1047 (s), 1020 (m), 997 (s). | |
| **HRMS**: (ESI) | Calcd for $(C_{10}H_{18}O_5+Na)^+$ | 241.1046 |
| | Found | 241.1050 |
| **TLC**: (50% ethyl acetate–hexanes) | $R_f$ = 0.63 (CAM) | |

β-anomer (major):

| | | |
|---|---|---|
| **$^{1}$H NMR**: (500 MHz, $CDCl_3$) | 4.67 (dd, 1H, *J* = 9.5, 2.7 Hz), 4.57 (d, 1H, *J* = 1.5 Hz), 4.15 (dq, 1H, *J* = 6.4, 1.5 Hz), 3.52 (s, 3H), 2.14 (s, 3H), | |

| | | |
|---|---|---|
| | 1.75–1.64 (m, 2H), 1.43 (s, 1H), 1.21 (s, 3H), 1.16 (d, 3H, $J$ = 6.4 Hz). | |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 170.9, 99.9, 74.1, 71.3, 67.9, 56.5, 39.6, 27.1, 20.8, 16.5. | |
| **FTIR**, $cm^{-1}$: (neat) | 3468 (br), 2936 (w), 1745 (s), 1447 (m), 1373 (m), 1234 (s), 1169 (m), 1153 (m), 1119 (m), 1053 (s), 1011 (s), 922 (m). | |
| **HRMS**: (ESI) | Calcd for $(C_{10}H_{18}O_5+Na)^+$ | 241.1046 |
| | Found | 241.1051 |
| **TLC**: (50% ethyl acetate–hexanes) | $R_f$ = 0.47 (CAM) | |
| **$[\alpha]_D$** | +13.3° ($c$ 0.40, $CHCl_3$), lit.[6] +14.5° ($c$ 0.5, $CHCl_3$). | |



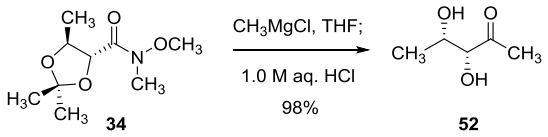

1-*O*-Acetyl Trioxacarcinose A (**48**).

Methyl trioxacarcinoside A **47** (200 mg, 0.916 mmol, 1 equiv, 1:1.7 mixture of α- and β- anomers, respectively) was treated with 1.0 M aqueous hydrochloric acid (9.16 mL, 9.16 mmol, 10.0 equiv) at 23 °C. After 16 h, sodium bicarbonate (924 mg, 11.0 mmol, 12 equiv) was added, and the product solution was saturated with sodium chloride. The mixture was extracted with ethyl acetate (3 × 50 mL). The organic layers were combined. The combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was dissolved in pyridine (13.9 mL). 4-Dimethylaminopyridine (170 mg, 1.39 mmol, 2.0 equiv) and acetic anhydride (69 μL, 0.73 mmol, 1.05 equiv) were added in sequence at 23 °C. After 15 h, a second portion of acetic anhydride (16 μL, 0.17 mmol, 0.25 equiv) was added. After 2.5 h, the product solution was partitioned between ethyl acetate (50 mL) and water (10 mL). The layers were separated. The organic layer was washed sequentially with 1.0 M aqueous hydrochloric acid solution (2 × 10 mL), saturated aqueous sodium bicarbonate solution (10 mL), then saturated aqueous sodium chloride solution (10 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (15% ethyl acetate–hexanes initially, grading to 40% ethyl acetate–hexanes) to provide 1-*O*-acetyl trioxacarcinose A (**48**) as a 1:3.4 mixture of α- and β-anomers, respectively (155 mg, 69%). For characterization purposes, a sample of the product (~50 mg) was further purified by flash-column chromatography (10% ethyl acetate–hexanes initially, grading

to 30% ethyl acetate–hexanes) to provide the pure β-anomer, which was characterized by $^{1}$H NMR, $^{13}$C NMR, IR, and HRMS.

β-**48**:

| | | |
|---|---|---|
| **$^{1}$H NMR**: (500 MHz, $CDCl_3$) | 5.99 (dd, 1H, *J* = 7.8, 4.9 Hz), 4.60 (s, 1H), 4.29 (q, 1H, *J* = 6.4 Hz), 2.25 (s, 1H), 2.13 (s, 3H), 2.08 (s, 3H), 1.76 (m, 2H), 1.20 (s, 3H), 1.13 (d, 3H, *J* = 6.4 Hz). | |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 170.7, 169.4, 91.8, 73.6, 71.0, 69.1, 38.4, 26.9, 21.1, 20.8, 16.4. | |
| **FTIR**, $cm^{-1}$: (neat) | 3482 (br), 2984 (m), 1734 (s), 1374 (m), 1233 (s), 1144 (s), 995 (s). | |
| **HRMS**: (ESI) | Calcd for $(C_{11}H_{18}O_6+Na)^+$ | 269.0996 |
| | Found | 269.0994 |
| **TLC**: (50% ethyl acetate–hexanes) | $R_f$ = 0.42 (CAM) | |




(3*R*,4*S*)-3,4-Dihydroxypentan-2-one (**52**).

Methylmagnesium chloride (3.0 M solution in tetrahydrofuran, 86.0 mL, 257 mmol, 3.0 equiv) was added over 15 min to an ice-cooled solution of (4*R*,5*S*)-*N*-methoxy-*N*,2,2,5-tetramethyl-1,3-dioxolane-4-carboxamide **34** (17.4 g, 86.0 mmol, 1 equiv) in tetrahydrofuran (570 mL). After 5 min, saturated aqueous ammonium chloride solution (80 mL) was added dropwise, and the reaction flask was allowed to warm to 23 °C. Aqueous hydrochloric acid solution (1.0 M, 570 mL) was added, a reflux condenser was affixed, and the reaction flask was heated at 60 °C. After 90 min of vigorous stirring, the reaction flask was allowed to cool to 23 °C. The product mixture was concentrated. Ethyl acetate (1 L) was added, and the suspension was dried over sodium sulfate. The dried suspension was filtered and the filtrate was concentrated to provide (3*R*,4*S*)-3,4-dihydroxypentan-2-one (**52**) as a colorless oil (9.89 g, 98%). Analytical data were in agreement with values previously reported.[60]

| | |
|---|---|
| **$^1$H NMR**: | 4.18 (m, 1H), 3.98 (dd, 1H, $J$ = 4.6, 2.3 Hz), 3.81 (d, 1H, |
| (500 MHz, $CDCl_3$) | $J$ = 4.6 Hz), 2.44 (d, 1H, $J$ = 9.2 Hz), 2.24 (s, 3H), 1.30 |
| | (d, 3H, $J$ = 6.4 Hz). |
| **$^{13}$C NMR**: | 208.3, 80.4, 67.9, 25.5, 20.0. |

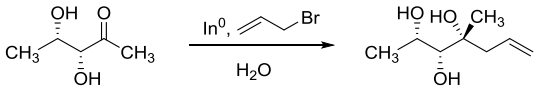
[60] Zörb, A.; Brückner, R. *Eur. J. Org. Chem.* **2010**, *2010*, 4785–4801.

(125 MHz, $CDCl_3$)

| | | |
|---|---|---|
| **FTIR**, $cm^{-1}$: | 3421 (br), 2934 (w), 1713 (s), 1360 (m), 1242 (m), 1134 | |
| (neat) | (s), 1074 (s), 1009 (m). | |
| **HRMS**: | Calcd for $(C_5H_{10}O_3+Na)^+$ | 141.0522 |
| (ESI) | Found | 141.0524 |

**TLC**: $R_f = 0.37$ (CAM)

(80% ethyl acetate–hexanes)




**52** **53**

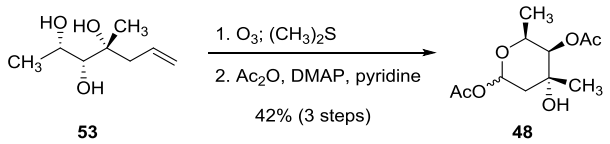
(2*S*,3*R*,4*R*)-4-Methylhept-6-ene-2,3,4-triol (**53**).

Allyl bromide (3.77 mL, 43.5 mmol, 1.5 equiv) was added to a mixture of (3*R*,4*S*)-3,4-dihydroxypentan-2-one **52** (3.43 g, 29.0 mmol, 1 equiv) and indium powder (5.00 g, 43.5 mmol, 1.5 equiv) in water (290 mL) at 23 °C. The reaction flask was sealed with a rubber septum. After 1 h of vigorous stirring, the product mixture was concentrated. Ethyl acetate (200 mL) was added, and the suspension was dried over sodium sulfate. The dried suspension was filtered and the filtrate was concentrated. A small sample of the product (~50 mg) was purified by flash-column chromatography (60% ethyl acetate–hexanes initially, grading to 100% ethyl acetate) and the purified product was characterized by $^{1}$H NMR, $^{13}$C NMR, IR, and HRMS.[61] The balance of the product, (2*S*,3*R*,4*R*)-4-methylhept-6-ene-2,3,4-triol (**53**), was used directly in the next reaction without purification.

**$^{1}$H NMR**: (500 MHz, DMSO-$d_6$) 5.88–5.79 (m, 1H), 5.01 (d, 1H, $J$ = 4.9 Hz), 4.99 (d, 1H, $J$ = 1.0 Hz), 4.45 (s, 1H), 4.40 (d, 1H, $J$ = 4.9 Hz), 4.18 (d, 1H, $J$ = 7.8 Hz), 3.95 (m, 1H), 2.91 (d, 1H, $J$ = 7.8 Hz), 2.23 (m, 2H), 1.08 (d, 3H, $J$ = 6.4 Hz), 1.06 (s, 3H).

[61] The product obtained in this procedure is a 15:1 mixture of diastereomers (epimeric tertiary alcohols); the major diastereomer is depicted in the equation above. The minor C4 epimer was carried through as an impurity to the stage of the acetylation reaction (vide infra), where the diastereomeric products were easily separated.

| | | |
|---|---|---|
| **$^{13}$C NMR**: | 135.5, 116.8, 76.8, 74.3, 65.5, 42.9, 24.1, 21.4. | |
| (125 MHz, DMSO-$d_6$) | | |
| **FTIR**, $cm^{-1}$: | 3379 (br), 2934 (m), 1377 (m), 1124 (s), 997 (s), 916 (s). | |
| (neat) | | |
| **HRMS**: | Calcd for $(C_8H_{16}O_3+Na)^+$ | 183.0992 |
| (ESI) | Found | 183.0982 |
| **TLC**: | $R_f$ = 0.25 (CAM) | |
| (5% methanol–dichloromethane) | | |

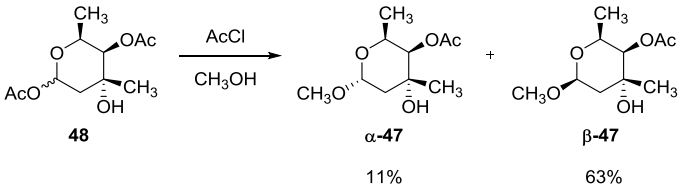



**53** **48**

1-*O*-Acetyl Trioxacarcinose A (**48**).

Ozone was bubbled through a solution of (2*S*,3*R*,4*R*)-4-methylhept-6-ene-2,3,4-triol **53** (1 equiv, see paragraph above) in methanol (290 mL) at –78 °C. After 30 min, oxygen was bubbled through the blue-green solution. After 10 min, methyl sulfide (21.3 mL, 290 mmol, 10.0 equiv) was added, and the reaction flask was allowed to warm to 23 °C. After 16 h, the solution was concentrated. The residue was dissolved in pyridine (290 mL). Acetic anhydride (5.76 mL, 61.0 mmol, ~2.1 equiv) and 4-dimethylaminopyridine (7.45 g, 61.0 mmol, ~2.1 equiv) were added in sequence at 23 °C. After 2 h, a second portion of acetic anhydride (2.74 mL, 29.0 mmol, ~1.0 equiv) was added. After 45 min, saturated aqueous sodium bicarbonate solution (100 mL) was added, and the product mixture was concentrated. The residue was partitioned between ethyl acetate (800 mL) and saturated aqueous sodium bicarbonate solution (200 mL). The layers were separated. The aqueous layer was extracted with ethyl acetate (800 mL). The organic layers were combined. The combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (15% ethyl acetate–hexanes initially, grading to 40% ethyl acetate–hexanes) to provide the product, 1-*O*-acetyl trioxacarcinose A (**48**), as a 1:12.1 mixture of α- and β-anomers, respectively (2.97 g, 42% yield over 3 steps). For characterization purposes, a sample of the product (~50 mg) was further purified by flash-column chromatography (10% ethyl acetate–hexanes initially, grading to 30% ethyl acetate–

hexanes) to provide the pure β-anomer, which was characterized by $^{1}H$ NMR, $^{13}C$ NMR, IR, and HRMS. See above for characterization data.




Conversion of 1-*O*-Acetyl Trioxacarcinose A (**48**) to Methyl Trioxacarcinoside A (**47**).

Acetyl chloride (289 μL, 4.06 mmol, 10.0 equiv) was added to a solution of 1-*O*-acetyl trioxacarcinose A **48** (100 mg, 406 μmol, 1 equiv, prepared from triol **53**) in methanol (4 mL) at 23 °C. After 10 min, sodium bicarbonate (341 mg, 4.06 mmol, 10.0 equiv) was added. The product mixture was filtered through a pad of Celite, washing with dichloromethane (40 mL). The filtrate was concentrated. The residue was purified by flash-column chromatography (20% ethyl acetate–hexanes initially, grading to 60% ethyl acetate–hexanes) to provide separately α- and β-methyl trioxacarcinoside A (**47**) (α-anomer: 10 mg, 11%; β-anomer: 56 mg, 63%). Analytical data for the synthetic substances were in agreement with those reported in the literature.[6]

α-anomer:

**$^1$H NMR**: (500 MHz, $CDCl_3$) 4.86 (d, 1H, *J* = 3.4 Hz), 4.70 (s, 1H), 4.32 (q, 1H, *J* = 6.4 Hz), 4.28 (s, 1H), 3.39 (s, 3H), 2.14 (s 3H), 1.93 (dd, 1H, *J* = 14.2, 3.9 Hz), 1.72 (dt, 1H, *J* = 14.7, 1.2 Hz), 1.13 (d, 3H, *J* = 6.4 Hz), 1.10 (s, 3H).

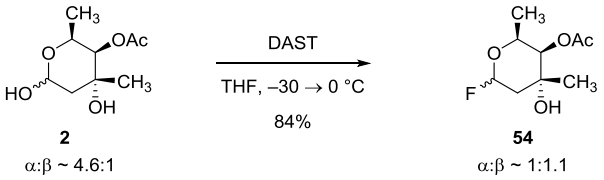
**$^{13}$C NMR**: (125 MHz, $CDCl_3$) 170.4, 99.1, 74.6, 68.8, 61.8, 55.2, 36.2, 25.7, 20.9, 16.6.

| | | |
|---|---|---|
| **FTIR**, $cm^{-1}$: | 3507 (br), 2938 (w), 1748 (s), 1375 (m), 1233 (s), 1121 | |
| (neat) | (s), 1082 (m), 1047 (s), 997 (s). | |
| **HRMS**: | Calcd for $(C_{10}H_{18}O_5+Na)^+$ | 241.1046 |
| (ESI) | Found | 241.1050 |
| **TLC**: | $R_f$ = 0.63 (CAM) | |
| (50% ethyl acetate–hexanes) | | |

β-anomer:

| | |
|---|---|
| **$^1$H NMR**: | 4.67 (dd, 1H, *J* = 9.5, 2.7 Hz), 4.57 (d, 1H, *J* = 1.5 Hz), |
| (500 MHz, $CDCl_3$) | 4.15 (dq, 1H, *J* = 6.4, 1.5 Hz), 3.52 (s, 3H), 2.14 (s, 3H), 1.75-1.64 (m, 2H), 1.43 (s, 1H), 1.21 (s, 3H), 1.16 (d, 3H, *J* = 6.4 Hz). |
| **$^{13}$C NMR**: | 170.9, 99.9, 74.1, 71.3, 67.9, 56.5, 39.6, 27.1, 20.8, 16.5. |
| (125 MHz, $CDCl_3$) | |
| **FTIR**, $cm^{-1}$: | 3468 (br), 2936 (w), 1745 (s), 1447 (m), 1373 (m), 1234 |

| | | |
|---|---|---|
| (neat) | (s), 1119 (m), 1053 (s), 1011 (s). | |
| **HRMS**: | Calcd for $(C_{10}H_{18}O_5+Na)^+$ | 241.1046 |
| (ESI) | Found | 241.1051 |
| **TLC**: | $R_f$ = 0.47 (CAM) | |
| (50% ethyl acetate–hexanes) | | |

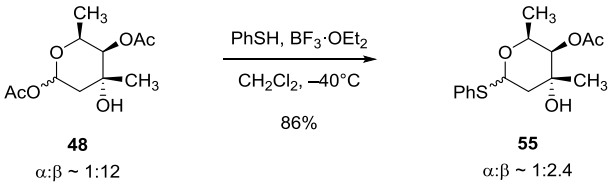



1-Fluorotrioxacarcinose A (**54**).

Diethylaminosulfur trifluoride (20 μL, 0.153 mmol, 1.2 equiv) was added to a solution of trioxacarcinose A (**2**) (26 mg, 0.127 mmol, 1 equiv) in tetrahydrofuran (2.5 mL) at –30 °C. The reaction flask was allowed to warm gradually to 0 °C over 30 min. Methanol (300 μL) was added and the reaction flask was allowed to warm to 23 °C. The mixture was partitioned between saturated aqueous sodium bicarbonate solution (1 mL) and ethyl acetate (10 ml). The layers were separated. The aqueous layer was extracted with ethyl acetate (2 × 10 mL). The organic layers were combined and the combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (30% ethyl acetate–hexanes) to provide 1-fluoro glycoside **54** as a 1:1.1 mixture of α and β anomers, respectively (22 mg, 84%).

| | |
|---|---|
| **$^1$H NMR**: | 5.79 (d, 1H, *J* = 53 Hz), 5.59 (ddd, 1H, *J* = 53, 9.3, 2.4 |
| (500 MHz, $CDCl_3$) | Hz), 4.78 (s, 1H), 4.59 (m, 2H), 4.27 (q, 1H, *J* = 6.8 Hz), |
| | 2.16 (s, 3H), 2.14 (s, 3H), 2.05–1.72 (m, 4H), 1.24 (s, |
| | 3H), 1.20 (d, 3H, *J* = 6.8 Hz), 1.17 (s, 3H), 1.16 (d, 3H, *J* |
| | = 6.8 Hz). |

**$^{19}$F NMR**: (375 MHz, $CDCl_3$) –124.25 (t, 1F, $J$ = 52.5 Hz), –135.02 (dd, 1F, $J$ = 52.5, 13.4 Hz).




**48**
α:β ~ 1:12

**55**
α:β ~ 1:2.4

1-Phenylthiotrioxacarcinoside A (**55**).

Boron trifluoride etherate (72 μL, 0.57 mmol, 1.4 equiv) was added to a solution of 1-*O*-acetyltrioxacarcinoside A (**48**) (100 mg, 0.41 mmol, 1 equiv, a 1:12 mixture of α- and β-anomers, respectively) and thiophenol (50 μL, 0.49 mmol, 1.2 equiv) in dichloromethane (8.1 mL) at –40 °C. After 5 min, saturated aqueous sodium bicarbonate solution (2 mL) was added rapidly. The cooling bath was removed and the reaction flask was allowed to warm to 23 °C. The mixture was partitioned between dichloromethane (50 mL) and saturated aqueous sodium bicarbonate solution (5 mL). The layers were separated. The organic layer was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (25% ethyl acetate–hexanes) to provide 1-phenylthiotrioxacarcinoside A (**55**), as a 2.4:1 mixture of β- and α-anomers, respectively (103 mg, 86%).

**$^1$H NMR**: (500 MHz, $CDCl_3$)

**α-anomer (minor):** 7.49 (d, *J* = 7.8 Hz, 2H), 7.31–7.22 (m, 3H), 5.64 (d, *J* = 6.4 Hz, 1H), 4.81 (q, *J* = 6.4 Hz, 1H), 4.74 (s, 1H), 2.58 (br, 1H), 2.35 (dd, *J* = 14.7, 6.4 Hz, 1H), 2.15 (s, 3H), 1.95 (m, 1H), 1.21 (s, 3H), 1.12 (d, *J* = 6.4 Hz, 3H).

| | |
|---|---|
| | **β-anomer (major):** 7.51 (d, *J* = 7.8 Hz, 2H), 7.31–7.22 (m, 3H), 5.19 (d, *J* = 11.7 Hz, 1H), 4.62 (s, 1H), 4.21 (q, *J* = 6.4 Hz, 1H), 2.15 (s, 3H), 1.93 (m, 1H), 1.82 (d, *J* = 13.7 Hz, 1H), 1.20 (s, 3H), 1.17 (d, *J* = 6.4 Hz, 3H). |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 170.8, 170.5, 136.4, 134.7, 130.7, 130.6, 128.8, 128.7, 126.9, 126.8, 83.4, 80.7, 74.4, 73.7, 70.6, 70.4, 69.1, 62.8, 39.5, 38.3, 27.0, 26.8, 20.8, 20.8, 17.0, 16.6. |
| **FTIR**, $cm^{-1}$: (neat) | 3493 (br), 2980 (m), 1743 (s), 1373 (m), 1233 (s), 1067 (s), 1007 (s). |
| **HRMS**: (ESI) | Calcd for $(C_{15}H_{20}O_4S+Na)^+$ 319.0975<br>Found 319.0987 |
| **TLC**: (50% ethyl acetate–hexanes) | $R_f$ = 0.71 (CAM) |

# Chapter 3

# Component-Based Syntheses of Trioxacarcin A and DC-45-A1

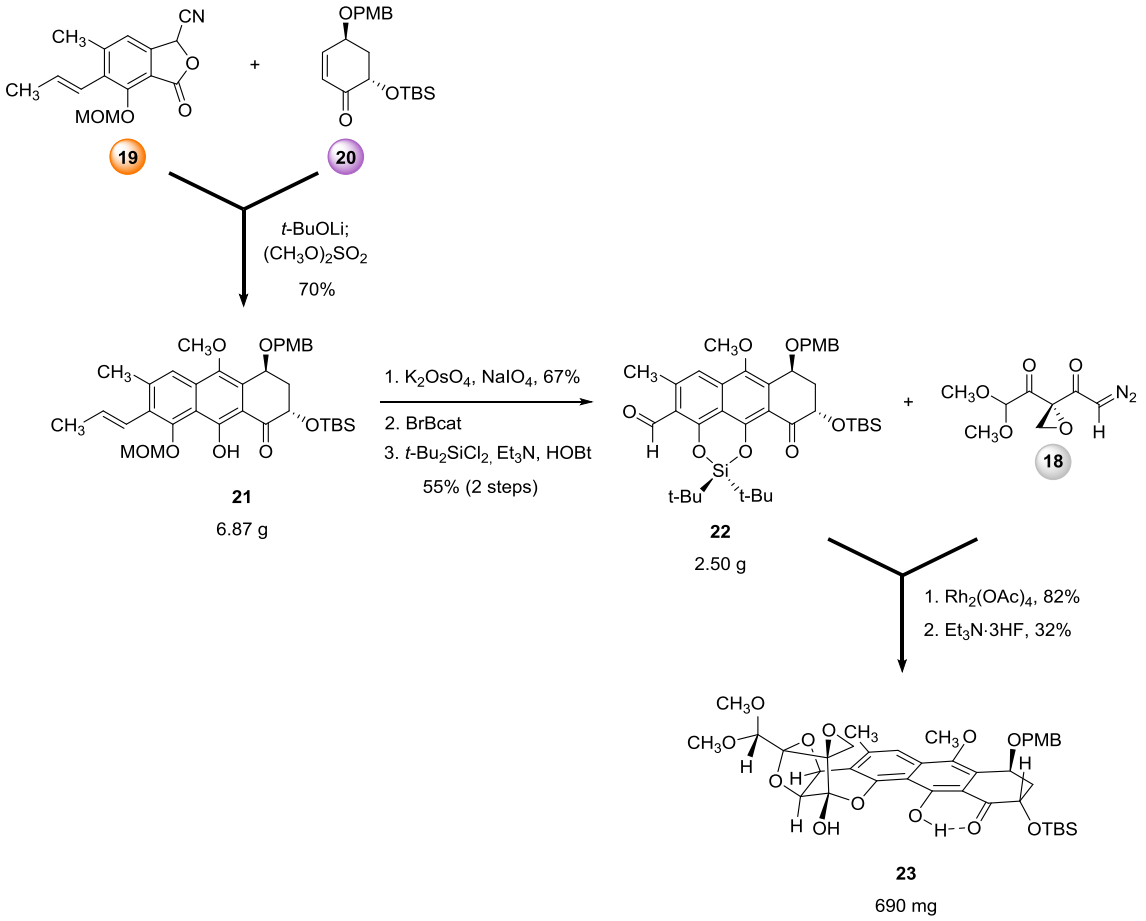
## Introduction

In this chapter, I present details of the research that led to the development of a component-based synthetic route to trioxacarcin A (**1**) and DC-45-A1 (**10**), both glycosylated natural products of the trioxacarcin class. In the first section, I describe the gram-scale execution of the route to differentially protected synthetic precursor **23** originally developed by graduate students Jakub Švenda and Nicholas Hill.[18] This route proceeds by the assembly of three components of similar synthetic complexity: epoxy diazo diketone **18**, cyanophthalide **19**, cyclohexenone **20**. I then discuss the development of α-selective glycosylation methodology for the coupling of a trioxacarcinose A residue to the C4 hydroxyl group of fully functionalized trioxacarcin core substrates. These studies enabled a three-step synthesis of the natural glycosylated trioxacarcin DC-45-A1 (**10**) from synthetic precursor **23**. Simultaneously, postdoctoral researcher Thomas Magauer developed a method for the α-selective introduction of trioxacarcinose B (**3**) to the C13 hemiketal group of the trioxacarcins.[62] In the final section, I describe the use of these two glycosylation methodologies in tandem to prepare bisglycosylated trioxacarcins containing both trioxacarcinose A (**2**) and trioxacarcinose B (**3**). The result of these efforts was a highly convergent synthesis of trioxacarcin A (**1**), proceeding in 11 steps from five components of similar complexity: epoxy diazo diketone **18**, cyanophthalide **19**, cyclohexenone **20**, trioxacarcinose A glycosyl donor **48**, and a trioxacarcinose B glycosyl donor **65**.

[62] Magauer, T.; Smaltz, D. J.; Myers, A. G. *Nat. Chem.* **2013**, *5*, 886–893.

**Large-Scale Synthesis of Differentially Protected DC-45-A2**

Before beginning glycosylation studies, it was necessary to prepare large quantities of synthetic precursor **23**, which represents a differentially protected form of the natural product DC-45-A2, the aglycon common to glycosylated trioxacarcins (see Scheme 3.1). We had anticipated that this precursor would serve directly as a substrate for the introduction of carbohydrates such as trioxacarcinose B (**3**) by glycosylation of the hemiketal hydroxyl group at position C13. Furthermore, after cleavage of the *p*-methoxybenzyl ether protective group, this precursor would provide a substrate for selective introduction of carbohydrates such as trioxacarcinose A (**2**) by glycosylation of the hydroxyl group liberated at position C4, which we expected to be more reactive than the C13 hemiketal. In earlier work, graduate students Jakub Švenda and Nicholas Hill prepared 48 mg of differentially protected DC-45-A2 (**23**) in a single batch.[18] We anticipated a need for larger quantities of this material for glycosylation studies directed toward the synthesis of DC-45-A1 (**10**) and trioxacarcin A (**1**), as well as for the synthesis of unnatural trioxacarcin analogs.




**Scheme 3.1.** Large-Scale Synthesis of Synthetic Precursor **23**, a Differentially-Protected Form of DC-45-A2.

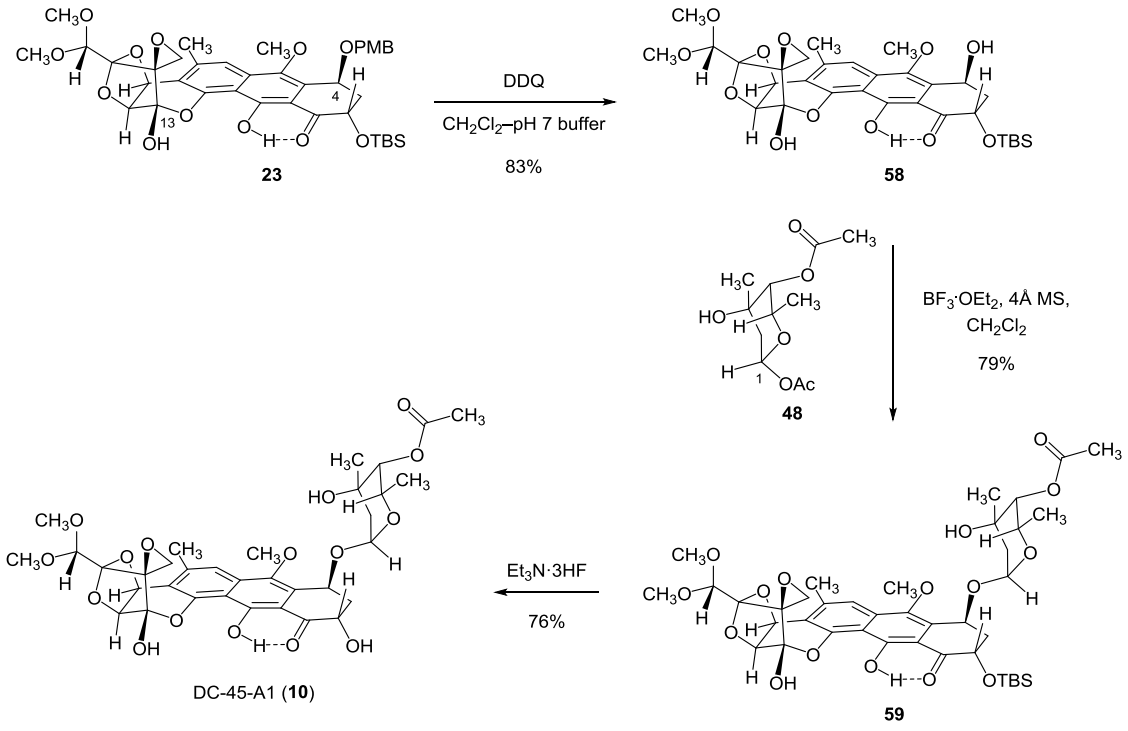
We were therefore led to scale the synthetic route to the differentially protected synthetic precursor **23**, and we observed increased yields for many steps upon scaling (cf. Scheme 1.3). These improvements combined with procedural refinements allowed us to prepare 690 mg of synthetic precursor **23** in diastereomerically pure form in a single batch, a more than tenfold scaling of the previous route (Scheme 3.1). Thus, coupling of cyanophthalide **19** and enone **20** in an anionic cyclization reaction furnished the dihydroanthracenone adduct **21** in 70% yield. Dihydroanthracenone **21** was transformed into aldehyde **22** (2.50 g) in a three-step sequence involving oxidative cleavage of the

disubstituted olefin (67% yield), removal of the methoxymethyl ether protective group, and silylation of the bisphenol function (55% yield over two steps). To conduct the key carbonyl ylide–aldehyde cycloaddition on a large scale, a solution of the epoxy diazo diketone component **18** (2.27 g, 3.0 equiv, 2.72 M in dichloromethane) was added by a motor-driven syringe pump over 6 h to a suspension of differentially protected aldehyde **22** (2.50 g, 1 equiv, 0.91 M in dichloromethane), rhodium(II) acetate (0.02 equiv), and powdered 4-Å molecular sieves at 23 °C. After filtration to remove molecular sieves and the rhodium catalyst, purification by flash-column chromatography on silica gel provided a mixture of the four possible diastereomeric cycloadducts (see Scheme 1.4) in 82% yield (2.59 g), and, separately, the aldehyde starting material in 15% yield. Separation of the individual diastereomeric cycloadducts at this stage was challenging; instead, it proved more convenient to remove the cyclic di-*tert*-butylsiloxane protective group first, after which only the endo diastereomers underwent spontaneous hemiketalization. Critically, we found that the two major endo diastereomers, which in previous smaller-scale runs had been separated by preparatory RP-HPLC,[18] could be separated by flash-column chromatography on silica gel deactivated with triethylamine (as the exo diastereomers do not undergo hemiketalization, they differ greatly in polarity from the endo diastereomers at this stage). In this manner we isolated 690 mg (32% yield) of synthetic precursor **23** in a single batch.

**Synthesis of DC-45-A1**

With a scalable route to large quantities of synthetic precursor **23**, we began to study the glycosidic coupling of an appropriately activated trioxacarcinose A derivative

to the benzylic hydroxyl group at C4 of the trioxacarcin skeleton. The substrate for glycosylation, benzylic alcohol **58**, was prepared in 83% yield by treatment of synthetic precursor **23** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in a mixture of dichloromethane and pH 7 aqueous phosphate buffer at 23 °C (Scheme 3.2).[18] We expected the benzylic C4 hydroxyl group revealed by this transformation to be more reactive in glycosylation reactions than either the C13 hemiketal group or the hydrogen-bonded phenol, neither of which were protected.




**Scheme 3.2.** Synthesis of DC-45-A1 (**10**) from Synthetic Precursor **23**.

In addition to the monoglycoside DC-45-A1 (**10**), the trioxacarcinose A residue is common to many of the naturally occurring trioxacarcins, including the bisglycoside

trioxacarcin A (**1**) and the trisglycoside LL-D49194α1 (**12**) (Figure 1.2). Trioxacarcinose A (**2**) contains a free hydroxyl group which lies in a 1,3-diaxial relationship with the α-glycosidic linkage present within each of these natural products. One early point of concern for glycosylation studies was the issue of whether to protect this alcohol, and if so how. It was easy to envision many protection schemes that would disfavor α-glycosylation through steric interactions, and less obvious how to functionalize this alcohol to promote the desired stereochemical outcome. Furthermore, trioxacarcinose A (**2**) is a 2-deoxy sugar and, lacking 2-substituents to direct the stereochemistry of anomeric bond formation, these frequently prove to be problematic substrates.[63]

I explored initial glycosidic couplings using 1-*O*-acetyl trioxacarcinose A (**48**). I found that when a mixture of aglycon **58** (1 equiv), glycosyl donor **48** (2 equiv, a 1:12 mixture of α- and β-anomers, respectively) and powdered 4-Å molecular sieves in dichloromethane at –40 °C was treated with boron trifluoride etherate (1.0 equiv) as promoter, the α-monoglycoside **59** was formed in 79% yield (23 mg, Scheme 3.2). The β-configured glycosylation product was not observed. Trimethylsilyl trifluoromethanesulfonate was also effective for the stereoselective coupling of 1-*O*-acetyltrioxacarcinose A (**48**) to substrate **58**, although the yield was lower. Thus, when a suspension of substrate **58** (1 equiv), 1-*O*-acetyltrioxacarcinose A (**48**, 2 equiv, a 1:12 mixture of α- and β-anomers, respectively), and powdered 4-Å molecular sieves in dichloromethane at –78 °C was treated with a catalytic quantity of trimethylsilyl trifluoromethanesulfonate (0.3 equiv), the α-monoglycoside **59** was formed in 52% yield, and the β-glycoside product was not observed. In neither of the above coupling reactions

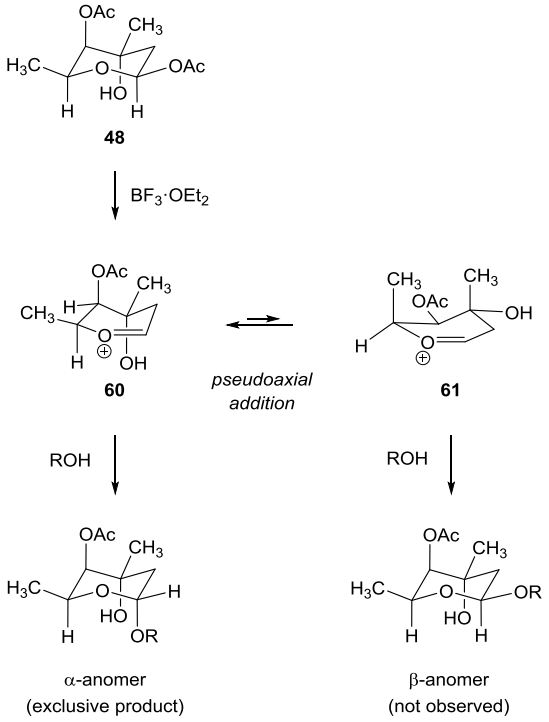
[63] (a) Veyrières, A.; Ernst, B.; Hart, G. W.; Sinaý, P., Special Problems in Glycosylation Reactions: 2-Deoxy Sugars. In *Carbohydrates in Chemistry and Biology*, Wiley-VCH Verlag GmbH: 2008; pp 367–405. (b) Gholap, S. L.; Woo, C. M.; Ravikumar, P.; Herzon, S. B. *Org. Lett.* **2009**, *11*, 4322–4325.

did I observe glycosylation of either the C13 hemiketal or the phenol. Furthermore, the success of these glycosidic couplings indicated that protection of the free 3-hydroxyl group present within the 1-*O*-acetyl glycoside **48** was unnecessary. In contrast to these successful couplings, attempts to introduce the trioxacarcinose A residue using the corresponding moisture- and acid-sensitive 1-fluoro glycoside derivative **54** (Scheme 2.9) did not afford any quantifiable amount of coupling products.

Removal of the *tert*-butyldimethylsilyl (TBS) ether of α-glycoside **59** was accomplished by treatment with a large excess of triethylamine trihydrofluoride (30 equiv) in acetonitrile at 23 °C for 16 h to provide DC-45-A1 (**10**) in 76% yield after purification by RP-HPLC (4.6 mg, Scheme 3.2). Analytical data obtained from the synthetic material were in agreement with values reported for the natural product.[3] The synthetic sample was also indistinguishable spectroscopically and chromatographically from an authentic sample (see Experimental Section).

Both conformational and stereoelectronic arguments can be advanced to explain the high α-selectivity observed in the glycosylation reaction of trioxacarcinose A. Lewis acid-promoted ionization of the trioxacarcinose A donor **48** forms an oxocarbenium ion intermediate that can in principle exist in two different half-chair conformations, depicted as **60** and **61** (Scheme 3.3). Two axial methyl groups would greatly impede axial addition of a glycosyl acceptor to the half-chair **61**, which is also disfavored stereoelectronically (as a consequence of the equatorial orientation of the 3-hydroxyl group), whereas axial addition to half-chair **60** is both sterically and stereoelectronically favored.[64]

[64] Romero, J. A. C.; Tabacco, S. A.; Woerpel, K. A. *J. Am. Chem. Soc.* **2000**, *122*, 168–169. (b) Ayala, L.; Lucero, C. G.; Romero, J. A. C.; Tabacco, S. A.; Woerpel, K. A. *J. Am. Chem. Soc.* **2003**, *125*, 15521–




**Scheme 3.3.** Rationalization of the Stereochemical Outcome of Glycosylation Reactions with Trioxacarcinose A Donor **48**.

A remarkable—and essential—feature of the glycosylation reaction of the trioxacarcinose A donor **48** is that it is compatible with the spiro epoxide function of the core substrate. Evidence suggests that the epoxy group efficiently alkylates only nucleophiles that benefit from $\pi$-stacking interactions with the aromatic core, such as a guanosine base of duplex DNA.[17]

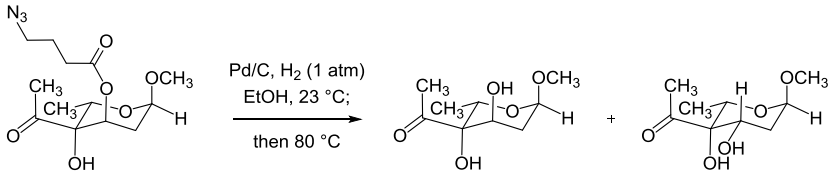
15528. (c) Chamberland, S.; Ziller, J. W.; Woerpel, K. A. *J. Am. Chem. Soc.* **2005**, *127*, 5322–5323. (d) Yang, M. T.; Woerpel, K. A. *J. Org. Chem.* **2009**, *74*, 545–553.

**Synthesis of Trioxacarcin A**

Having developed a method for the coupling of trioxacarcinose A to the C4 hydroxyl group of trioxacarcin aglycon substrates such as **58**, we turned our attention to the introduction of trioxacarcinose B (**3**) to the sterically hindered C13 hemiketal. A variety of anomerically activated derivatives of trioxacarcinose B are available from a short and practical sequence developed by postdoctoral researcher Thomas Magauer.[65] Attempts to glycosylate differentially protected aglycon substrate **23** with trioxacarcinose B derivatives bearing a free 3-hydroxyl group were not successful, so we elected to protect that hydroxyl group. In evaluating potential protective groups, we prioritized those which might minimize a potential 1,3-diaxial steric interaction with an incoming nucleophile, for we anticipated that such an interaction would serve to disfavor the required α-glycosidic bond formation. For this reason we prioritized ester protective groups. Because the trioxacarcinose A residue present in many natural trioxacarcins bears an acetate ester, initially we sought to use an ester group other than acetate to protect position C3 of the trioxacarcinose B donor, to avoid a problem of redundant functionality. In a model study, Thomas Magauer evaluated the use of a 4-azidobutyryl ester as a differentiated ester protective group. Accordingly, hydrogenolysis of model substrate **62** (Pd/C, 1 atm $H_2$, ethanol, 23 °C), followed by brief heating at 80 °C to promote cleavage of the intermediate 4-aminobutyryl ester, produced a complex mixture of products including the desired C3-alcohol (**63**) and its epimer (3-*epi*-**63**). We reasoned that 3-*epi*-**63** likely arose from a retroaldol fragmentation–aldol cyclization sequence.

[65] Magauer, T.; Myers, A. G. *Org. Lett.* **2011**, *13*, 5584–5587.

This not only indicated that a 4-azidobutyryl ester was unsuitable in this context, but also suggested that earlier attempts at glycosidic coupling of trioxacarcinose B derivatives bearing a free 3-hydroxyl group failed due to decomposition of the sugar by pathways initiated by retroaldol fragmentation.

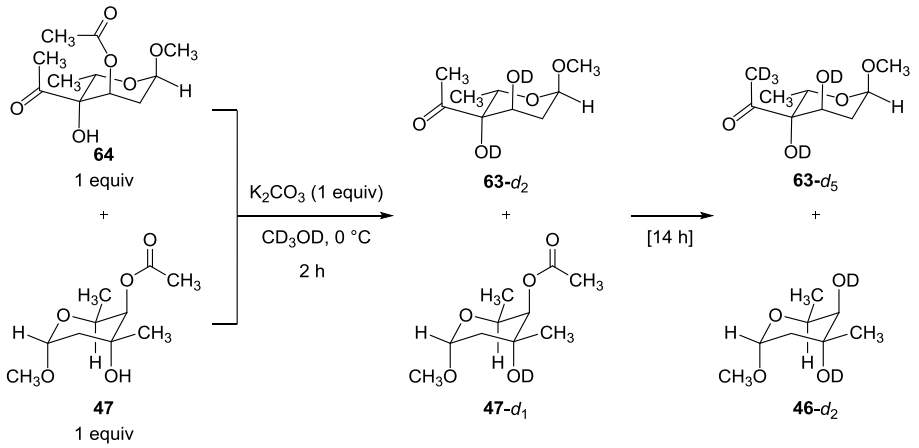



**62** **63** 3-*epi*-**63**

**Scheme 3.4.** Attempted Deprotection of 4-Azidobutyryl Ester **62**.

At this stage we considered carefully whether it was necessary to differentiate the 3-hydroxyl protective group of the trioxacarcinose B residue from the acetate ester within the trioxacarcinose A residue. We conducted a competition experiment suggesting that such differentiation was unnecessary. Thus, a mixture of methyl α-3-*O*-acetyl trioxacarcinoside B (**64**) (1 equiv) and methyl α-trioxacarcinoside A (**47**) (1 equiv) in $CD_3OD$ was treated with potassium carbonate (1 equiv) at 0 °C, and the mixture was monitored by $^1H$ NMR analysis (500 MHz). Within 2 h, complete deprotection of the 3-*O*-acetyl protective group of the trioxacarcinose B residue occurred to give methyl α-trioxacarcinoside B (**63**), and the acetate ester within the methyl α-trioxacarcinoside A residue was unaffected (Scheme 3.5). (Complete deprotection of both esters occurred on prolonged stirring at 0 °C.) We rationalized the difference in stability between these two acetate esters based on their different steric environments: Whereas the α-trioxacarcinose

A ester is flanked on both sides by equatorial methyl groups, the 3-*O*-acetyl ester group of the trioxacarcinose B residue has only one flanking equatorial group.

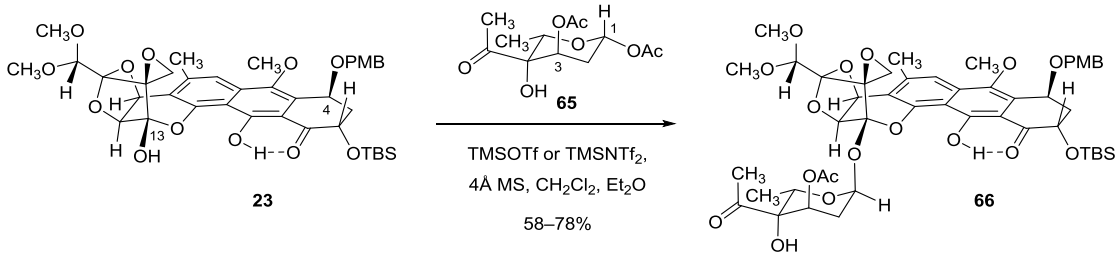



**Scheme 3.5.** Simultaneous Methanolysis of Methyl α-3-*O*-Acetyl Trioxacarcinoside B (**64**) and Methyl α-Trioxacarcinoside A (**47**).

With the above evidence that 1,3-di-*O*-acetyl trioxacarcinose B (**65**) could be a viable glycosyl donor for the preparation of fully synthetic bisglycoside trioxacarcins such as trioxacarcin A, postdoctoral researcher Thomas Magauer developed conditions for the coupling of this activated sugar to differentially protected synthetic precursor **23**. In an early successful coupling, a suspension of crushed, activated 4-Å molecular sieves, aglycon substrate **23** (1 equiv., 0.18 mM) and a large excess of donor **65** (30 equiv) in dichloromethane–ether at –78 °C was treated with excess TMSOTf (20 equiv)[66] to furnish the α-glycoside **66** as the sole product in 78% yield (30 mg).[62] By increasing the concentration of substrate **23**, the number of equivalents of donor **65** and Lewis acid that

[66] (a) Kimura, Y.; Suzuki, M.; Matsumoto, T.; Abe, R.; Terashima, S. *Chem. Lett.* **1984**, 501–504. (b) Kimura, Y.; Suzuki, M.; Matsumoto, T.; Abe, R.; Terashima, S. *Chem. Soc. Jpn* **1986**, *59*, 423–431.

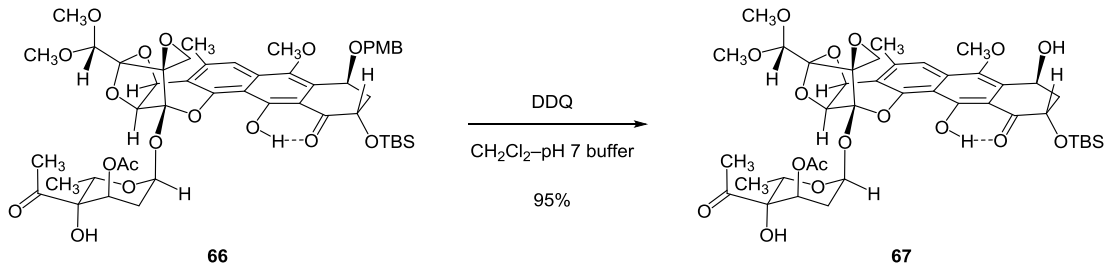
were necessary to achieve full conversion was reduced (e.g., 8.5 equiv. **65**, 58% yield, 120 mg, employing $TMSNTf_2$ as Lewis acid).[62]




**23** **65** **66**

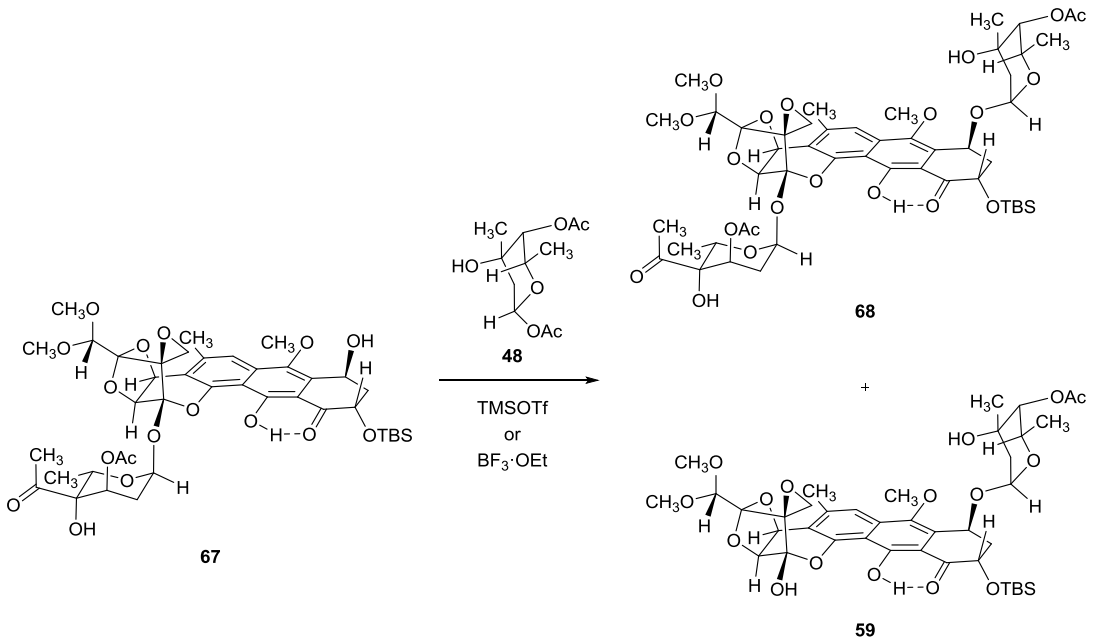
**Scheme 3.6.** Synthesis of Trioxacarcinose B Glycoside (**66**)[62]

With methods to independently couple both trioxacarcinose A (**2**) and trioxacarcinose B (**3**) to fully functionalized aglycon substrates, it remained to coordinate the two couplings to prepare trioxacarcin A (**1**). In principle, the two glycosidic couplings could be conducted in either possible sequence, but in practice it was necessary to conduct the coupling with trioxacarcinose B first, for when we attempted to glycosylate the trioxacarcinose-A containing monoglycoside **59** (see Scheme 3.2) with 1,3-di-*O*-acetyl trioxacarcinose B (**65**) using TMSOTf as promoter, conditions that were successful for the glycosylation of aglycon **23**, only products arising from cleavage of the existing α-glycosidic linkage were observed.




**Scheme 3.7.** Cleavage of the PMB Ether within Glycoside **66**.

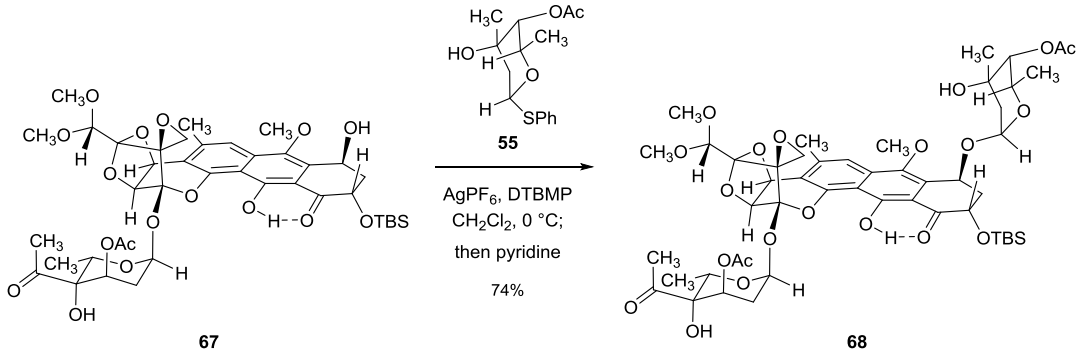
To evaluate the alternative sequence, the PMB ether within monoglycoside **66** was cleaved (DDQ, 1.1 equiv., 95% yield) to provide benzylic alcohol **67** (Scheme 3.7). Attempted glycosylations of **67** with 1-*O*-acetyl trioxacarcinose A (**48**) using either TMSOTf or boron trifluoride etherate as Lewis-acidic promoter provided the desired bisglycoside product **68** alongside a byproduct arising from cleavage of the trioxacarcinose B residue, namely monoglycoside **59** (Scheme 3.8).




**Scheme 3.8.** Attempted Lewis Acid-Promoted Glycosylation of Monoglycoside **67** with 1-*O*-Acetyltrioxacarcinose A (**48**).

We were therefore led to explore orthogonal conditions for glycosylation and considered the use of 1-phenylthiotrioxacarcinoside A (**55**, see Scheme 2.9) as an alternative coupling partner. Addition of silver hexafluorophosphate (6.0 equiv) to a mixture of substrate **67** (65 mg, 1 equiv), 1-phenylthiotrioxacarcinoside A (**55**, 3.0 equiv, a 1:2.4 mixture of α- and β-anomers, respectively), 2,6-di-*tert*-butyl-4-methylpyridine (8.0 equiv) and powdered 4-Å molecular sieves in dichloromethane at 0 °C led to complete glycosylation within 1 hour (Scheme 3.9).[67] The α,α-bisglycoside **68** was obtained as a greenish-yellow foam after purification by RP-HPLC (58 mg, 74%). The β-glycoside was not observed, and, critically, no products resulting from cleavage of the trioxacarcinose B residue were detected.

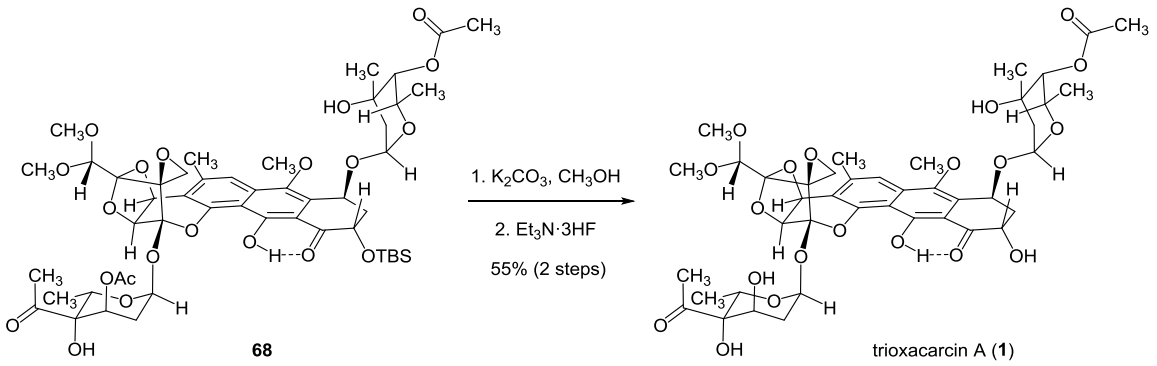
[67] (a) Lear, M. J.; Yoshimura, F.; Hirama, M. *Ang. Chem. Int. Ed.* **2001**, *40*, 946–949. (b) Ren, F.; Hogan, P. C.; Anderson, A. J.; Myers, A. G. *J. Am. Chem. Soc.* **2007**, *129*, 5381–5383.




**Scheme 3.9.** Coupling of Benzylic Alcohol Monoglycoside **67** with 1-Phenylthiotrioxacarcinoside A (**55**)

From bisglycoside **68**, the cleavage of two protective groups, namely the C2 *tert*-butyldimethylsilyl ether and the trioxacarcinose B acetate ester, was required to prepare trioxacarcin A (**1**). Selective cleavage of the trioxacarcinose B acetate ester protective group occurred on exposure of the α,α-bisglycoside **68** to potassium carbonate for one hour in ice-cold methanol (Scheme 3.10); higher temperatures led to decomposition. $^{1}$H NMR analysis of the yellow-orange residue established that methanolysis of the trioxacarcinose B acetate ester had occurred cleanly, and showed no evidence of any competing cleavage of the trioxacarcinose A acetate ester, as anticipated from model studies (see Scheme 3.5). The product was not purified, but was subjected to immediate deprotection with triethylamine–trihydrofluoride in acetonitrile at 23 °C (16 hours). After isolation by RP-HPLC, pure trioxacarcin A (**1**) was obtained as an orange-red powder (23 mg, 55% yield over two steps). Analytical data obtained from the synthetic material were in agreement with values reported for the natural product.[2,6] The synthetic sample was also indistinguishable spectroscopically and chromatographically from an authentic sample. We observed that trioxacarcin A, like its precursor **68**, is unstable towards a base

in alcoholic solvents; decomposition is evidenced by the formation of a deep burgundy-red solution (solutions of pure trioxacarcin A in ethanol and chloroform are typically orange).




**Scheme 3.10.** Synthesis of Trioxacarcin A (**1**)

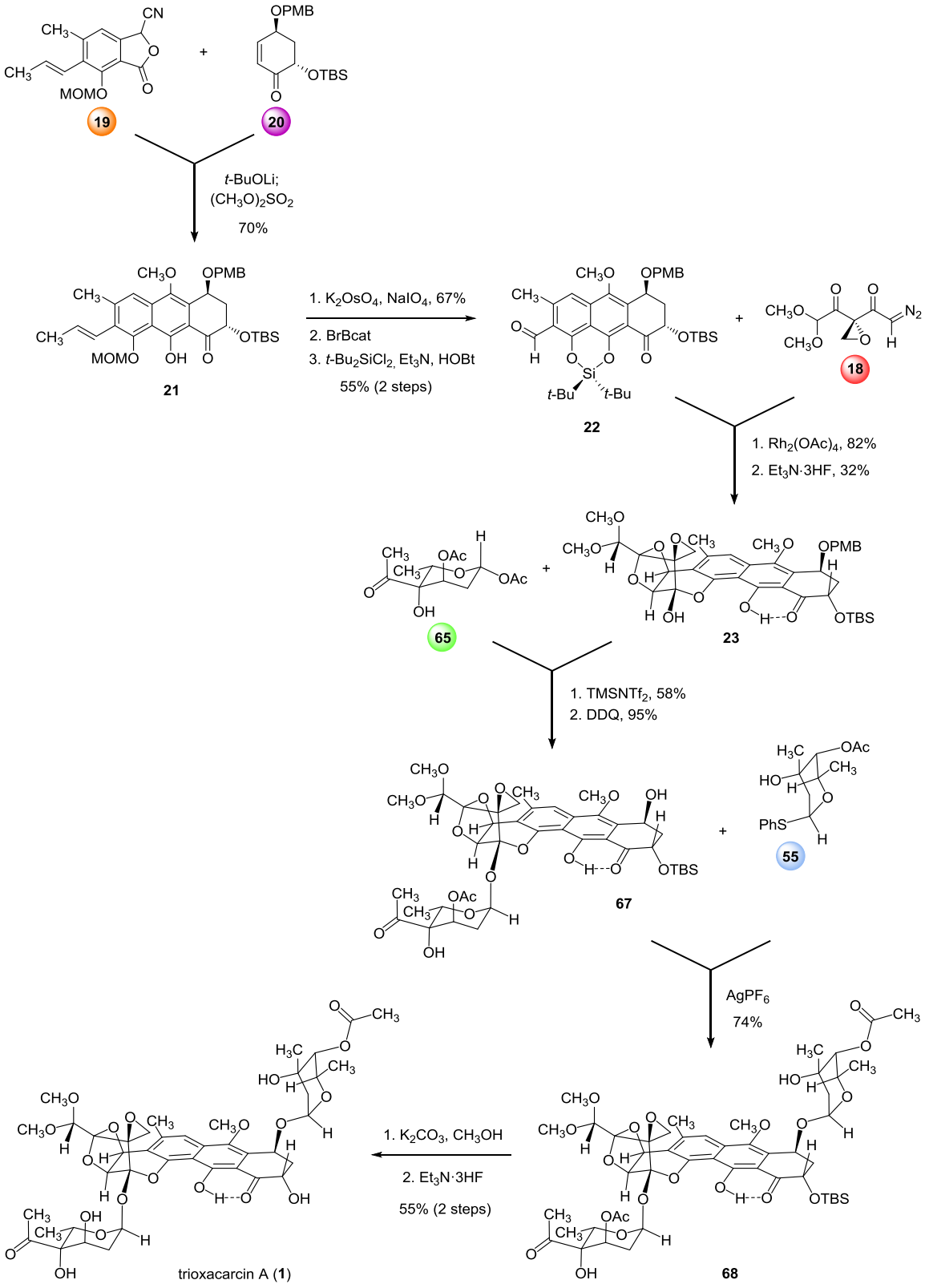
## Conclusion

In this chapter, I have described the development of component-based synthetic routes to trioxacarcin A (**1**) and DC-45-A1 (**10**). These routes represent the first syntheses of natural, glycosylated trioxacarcins. Both rely on the late-stage glycosidic coupling of an activated form of trioxacarcinose A (**48** or **55**) to a fully functionalized trioxacarcin core substrate.

The synthetic route to trioxacarcin A (**1**) assembles five components of similar synthetic complexity: epoxy diazo diketone **18**, cyanophthalide **19**, cyclohexenone **20**, trioxacarcinose A donor **55**, and trioxacarcinose B donor **65**. Each of these components in turn was prepared in multigram quantities by a short, practical sequence. Starting from

the five components, trioxacarcin A (**1**) is prepared in 11 linear steps, four of which are convergent coupling reactions. The route is summarized in Scheme 3.11, with convergent couplings denoted by branched vertical arrows and other linear steps by horizontal arrows. A graphical schematic of the route is shown in Figure 3.1 and emphasizes the convergent, component-based nature of the sequence. Within the schematic, synthetic intermediates involved in convergent coupling reactions are represented with circles, and each chemical step is represented by an arrow.

One key advantage of the component-based assembly strategy pursued for trioxacarcin A (**1**) and DC-45-A1 (**10**) is that deep-seated structural modifications can be introduced relatively rapidly by modification of the components. Modification of more than one component simultaneously leads to multiplicative expansion of the set of structures available for study. In Chapter 4 I describe the synthesis of a variety of novel trioxacarcin analogs by variation of the components, both individually and in combination, as well as by late-stage modification. Many of these analogs would be inaccessible by other means, demonstrating the advantages of a convergent, component-based synthetic sequence for the assembly of a target of interest.




**Scheme 3.11.** Component-Based Synthetic Route to Trioxacarcin A (**1**)

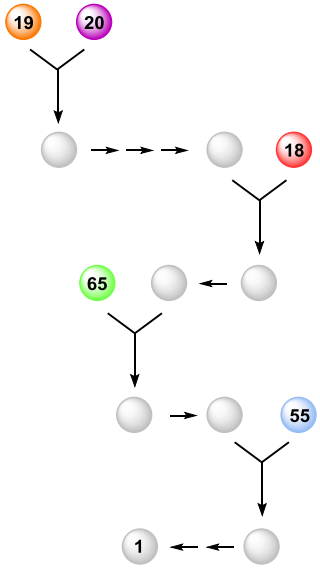



**Figure 3.1.** Graphical Schematic Representing the Synthetic Route to Trioxacarcin A (**1**).

## Experimental Section

*General Experimental Procedures*

All reactions were performed in round-bottom flasks fitted with rubber septa under a positive pressure of argon, unless otherwise noted. Air- and moisture-sensitive liquids were transferred by syringe or stainless steel cannula. Organic solutions were concentrated by rotary evaporation (house vacuum, ca. 25−40 Torr) at ambient temperature, unless otherwise noted. Analytical thin-layer chromatography (TLC) was performed using glass plates pre-coated with silica gel (0.25 mm, 60 Å pore-size, 230−400 mesh, Merck KGA) impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light, then were stained with either an aqueous sulfuric acid solution of ceric ammonium molybdate (CAM), an acidic solution of *p*-anisaldehyde in ethanol (Anis), or an aqueous sodium hydroxide–potassium carbonate solution of potassium permanganate ($KMnO_4$) followed by brief heating on a hot plate. Flash-column chromatography was performed as described by Still et al.,[52] employing silica gel (60 Å, 32-63 μm, standard grade, Dynamic Adsorbents, Inc.).

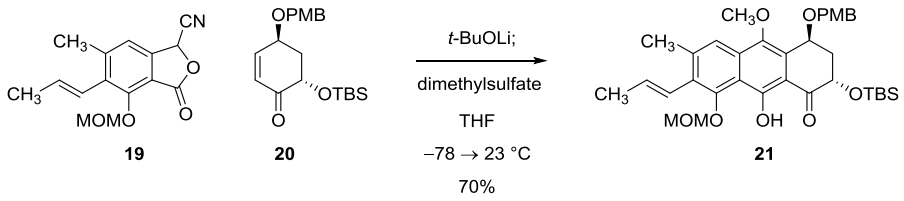
*Materials*

Commercial solvents and reagents were used as received with the following exceptions. Tetrahydrofuran, dichloromethane, benzene, toluene, and ether were purified by the method of Pangborn et al.[53] 4-Å molecular sieves (powder, Aldrich) were stored in a laboratory oven at 140 °C and were allowed to cool in a desiccator immediately before use.

*Instrumentation*

Proton magnetic resonance ($^1$H NMR) spectra were recorded on Varian INOVA 500 (500 MHz) or 600 (600 MHz) NMR spectrometers at 23 °C. Proton chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to residual protium in the NMR solvent ($CHCl_3$, δ 7.26). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances, br = broad, app = apparent), integration, and coupling constant (*J*) in Hertz. Carbon nuclear magnetic resonance spectra ($^{13}$C NMR) were recorded on Varian INOVA 500 (125 MHz) NMR spectrometers at 23 °C. Carbon chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to the carbon resonances of the NMR solvent ($CDCl_3$, δ 77.0). Infrared (IR) spectra were obtained using a Shimadzu 8400S FT-IR spectrometer. Data are represented as follows: frequency of absorption ($cm^{-1}$), intensity of absorption (s = strong, m = medium, w = weak, br = broad). Optical rotations were measured on a Jasco DIP-0181 digital polarimeter with a sodium lamp and are reported as follows: $[\alpha]^{T[^\circ C]}_{\lambda}$ (c = g/100 mL, solvent). Circular dichroism spectra were obtained using a Jasco J-710 spectropolarimeter. High-resolution mass spectra were obtained at the Harvard University Mass Spectrometry Facility. High performance liquid chromatography purifications were performed using an Agilent Technologies 1200 Series preparative HPLC system.

*(For clarity, intermediates that have not been assigned numbers in the text are numbered sequentially in the Experimental Section beginning with* **69***.)*

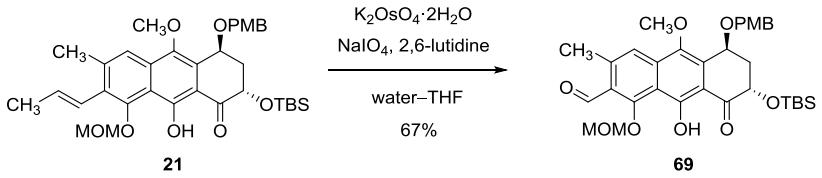



(*E*)-7-Propenyl-3,4-dihydroanthracen-1-one (**21**).[18]

Lithium *tert*-butoxide (1.0 M solution in tetrahydrofuran, 47.5 mL, 47.5 mmol, 3.0 equiv) was added to a solution of (*E*)-4-(methoxymethoxy)-6-methyl-3-oxo-5-(prop-1-enyl)-1,3-dihydroisobenzofuran-1-carbonitrile **19** (4.33 g, 15.8 mmol, 1 equiv) in tetrahydrofuran (86 mL) at –78 °C. After 5 min, a solution of (4*S*,6*S*)-6-(*tert*-butyldimethylsilyloxy)-4-(4-methoxybenzyloxy)cyclohex-2-enone **20** (5.74 g, 15.83 mmol, 1.0 equiv) in tetrahydrofuran (86 mL) was added by cannula. The reaction flask was allowed to warm to –20 °C over 3 h, then dimethylsulfate (13.6 mL, 142 mmol, 9.0 equiv) was added. The reaction flask was allowed to warm to 23 °C over 2 h. After an additional 2 h, the reaction mixture was partitioned between saturated aqueous ammonium chloride solution (500 mL) and ethyl acetate (1 L). The layers were separated. The organic layer was washed sequentially with water (500 mL) then saturated aqueous sodium chloride solution (500 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (hexanes initially, grading to 5% ethyl acetate–hexanes) to provide the product, (*E*)-7-propenyl-3,4-dihydroanthracen-1-one (**21**), as an orange foam (6.87 g, 70%).[18]

**$^1$H NMR**: 14.89 (s, 1H), 7.63 (s, 1H), 7.28 (d, 2H, $J$ = 8.5 Hz), 6.87

| | | |
|---|---|---|
| (500 MHz, $CDCl_3$) | (d, 2H, *J* = 8.2 Hz), 6.53 (d, 1H, *J* = 16.3 Hz), 6.11 (dq, 1H, *J* = 16.2, 6.4 Hz), 5.2 (m, 1H), 5.09 (d, 1H, *J* = 6.2 Hz), 5.04 (d, 1H, *J* = 6.2 Hz), 4.99 (dd, 1H, *J* = 12.6, 5.3 Hz), 5.78 (d, 1H, *J* = 11.0 Hz), 4.58 (d, 1H, *J* = 11.0 Hz), 3.85 (s, 3H), 3.79 (s, 3H), 3.59 (s, 3H), 2.72 (ddd, 1H, *J* = 13.3, 4.8, 3.2 Hz), 2.50 (s, 3H), 2.18 (m, 1H), 1.97 (d, 3H, *J* = 6.4 Hz), 0.98 (s, 9H), 0.25 (s, 3H), 0.18 (s, 3H). | |
| **$^{13}$C NMR**: | 203.7, 161.4, 159.2, 153.8, 144.8, 141.2, 133.1, 132.4, | |
| (125 MHz, $CDCl_3$) | 131.7, 130.2, 129.5, 125.4, 125.1, 119.1, 188.8, 113.8, 108.4, 101.3, 70.8, 69.4, 68.8, 62.8, 57.9, 55.3, 36.6, 25.9, 22.2, 19.3, 18.6, –4.4, –5.3. | |
| **FTIR**, $cm^{-1}$: | 2951 (w), 2930 (w), 1611 (m), 1514 (w), 1443 (w), 1381 | |
| (neat) | (w), 1362 (m), 1248 (m), 1155 (m), 1124 (m), 1040 (s), 1003 (m), 928 (m), 872 (m), 835 (m), 779 (m). | |
| **HRMS**: | Calcd for $(C_{35}H_{46}O_8Si+Na)^+$ | 645.2854 |
| (ESI) | Found | 645.2854 |
| **TLC**: | $R_f$ = 0.36 (CAM) | |
| (17% ethyl acetate–hexanes) | | |

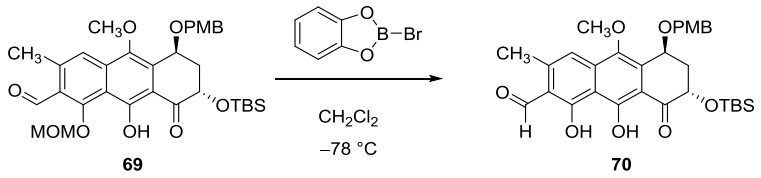



7-Formyl-3,4-dihydroanthracen-1-one (**69**)[18]

2,6-Lutidine (2.57 mL, 22.1 mmol, 2.0 equiv) was added to an ice-cooled solution of (*E*)-7-propenyl-3,4-dihydroanthracen-1-one **21** (6.87 g, 11.0 mmol, 1 equiv), potassium osmate dihydrate (203 mg, 0.552 mmol, 0.05 equiv), and sodium periodate (9.44 g, 44.1 mmol, 4.0 equiv) in a mixture of tetrahydrofuran (160 mL) and water (80 mL). After 5 min, the cooling bath was removed and the reaction flask was allowed to warm to 23 °C. After 2.5 h, the reaction mixture was partitioned between water (600 mL), ethyl acetate (1.2 L), and hexanes (600 mL). The layers were separated. The organic layer was washed with water (600 mL) then saturated aqueous sodium chloride solution (600 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (10% ethyl acetate–hexanes initially, grading to 15% ethyl acetate–hexanes) to provide the product, 7-formyl-3,4-dihydroanthracen-1-one (**69**), as an orange foam (4.52 g, 67%).[18]

| | |
|---|---|
| **$^1$H NMR**: | 14.96 (s, 1H), 9.64 (s, 1H), 7.64 (s, 3H), 7.28 (d, 2H, *J* = |
| (500 MHz, $CDCl_3$) | 8.5 Hz), 6.87 (d, 2H, *J* = 8.5 Hz), 5.29–5.20 (m, 3H), |
| | 5.00 (dd, 1H, *J* = 12.2, 5.1 Hz), 4.70 (d, 1H, *J* = 11.0 |
| | Hz), 4.60 (d, 1H, *J* = 11.0 Hz), 3.86 (s, 3H), 3.80 (s, 3H), |
| | 3.61 (s, 3H), 2.74 (s, 3H), 2.73 (m, 1H), 2.19 (m, 1H), |

| | | |
|---|---|---|
| | 0.98 (s, 9H), 0.26 (s, 3H), 0.18 (s, 3H). | |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 204.2, 193.3, 163.2, 161.6, 159.4, 144.7, 141.8, 137.0, 129.9, 129.5, 127.6, 120.7, 118.1, 113.9, 109.2, 102.8, 71.1, 69.3, 68.6, 62.9, 58.4, 55.3, 36.3, 25.8, 22.3, 18.5, –4.5, –5.3. | |
| **FTIR**, $cm^{-1}$: (neat) | 2953 (w), 2930 (w), 2857 (w), 1686 (m), 1611 (s), 1514 (m), 1385 (m), 1364 (m), 1246 (s), 1153 (s), 1042 (s), 1011 (m), 930 (m), 872 (m), 837 (m), 779 (m). | |
| **HRMS**: (ESI) | Calcd for $(C_{33}H_{42}O_9Si+Na)^+$ | 633.2490 |
| | Found | 633.2464 |
| **TLC**: (10% ethyl acetate–hexanes) | $R_f$ = 0.18 (CAM) | |

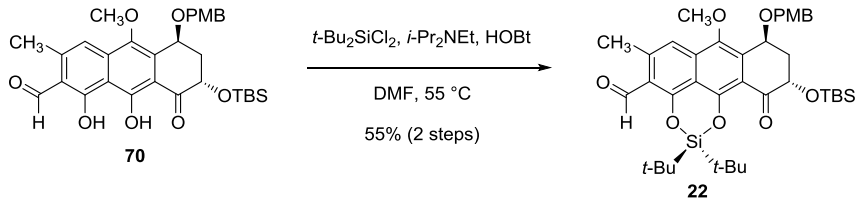



8,9-Dihydroxy-7-formyl-3,4-dihydroanthracen-1-one (**70**).[18]

A solution of *B*-bromocatecholborane (2.54 g, 12.8 mmol, 2.0 equiv) in dichloromethane (94 mL) was added to a solution of 7-formyl-3,4-dihydroanthracen-1-one **69** (3.90 g, 6.39 mmol, 1 equiv) in dichloromethane (94 mL) at –78 °C. After 50 min, the reaction mixture was diluted with saturated aqueous sodium bicarbonate solution (300 mL) and dichloromethane (500 mL). The cooling bath was removed, and the partially frozen mixture was allowed to warm to 23 °C. The biphasic mixture was diluted with 0.2 M aqueous sodium hydroxide solution (1.25 L). The layers were separated. The dark-purple aqueous layer was extracted with dichloromethane (2 × 1 L). The organic layers were combined. The combined solution was washed sequentially with 0.1 M aqueous hydrochloric acid solution (500 mL), water (3 × 500 mL), then saturated aqueous sodium chloride solution (500 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the product, 8,9-dihydroxy-7-formyl-3,4-dihydroanthracen-1-one (**70**), as a yellow foam.[18] Attempts to purify **70** by flash-column chromatography led to decomposition.

**$^1$H NMR**: (500 MHz, $CDCl_3$) 15.58 (br s, 1H), 10.51 (s, 1H), 7.28 (d, 2H, $J$ = 8.7 Hz), 7.25 (s, 1H), 6.87 (d, 2H, $J$ = 8.7 Hz), 5.16 (m, 1H), 4.99 (dd, 1H, $J$ = 12.3, 5.2 Hz), 4.68 (d, 1H, $J$ = 11.1 Hz), 4.59 (d, 1H, $J$ = 10.7 Hz), 3.84 (s, 3H), 3.80 (s, 3H), 2.73

| | | |
|---|---|---|
| | (s, 3H), 2.71 (m, 1H), 2.18 (m, 1H), 0.97 (s, 9H), 0.25 (s, 3H), 0.18 (s, 3H). | |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 203.9, 192.7, 166.5, 163.6, 159.4, 144.8, 142.7, 137.7, 130.7, 129.9, 129.5, 114.8, 114.2, 113.9, 108.5, 71.1, 69.0, 68.6, 62.8, 55.3, 36.5, 25.8, 21.0, 18.5, –4.5, –5.3. | |
| **FTIR**, $cm^{-1}$: (neat) | 3329 (w, br), 2953 (m), 2930 (m), 2857 (m), 1682 (m), 1614 (s), 1514 (m) 1391 (m) 1246 (s), 1153 (s), 1049 (m), 1034 (m) 870 (m), 837 (s). | |
| **HRMS**: (ESI) | Calcd for $(C_{31}H_{38}O_8Si+H)^+$ | 567.2409 |
| | Found | 567.2398 |
| **TLC**: (30% ethyl acetate–hexanes) | $R_f$ = 0.22 (CAM) | |

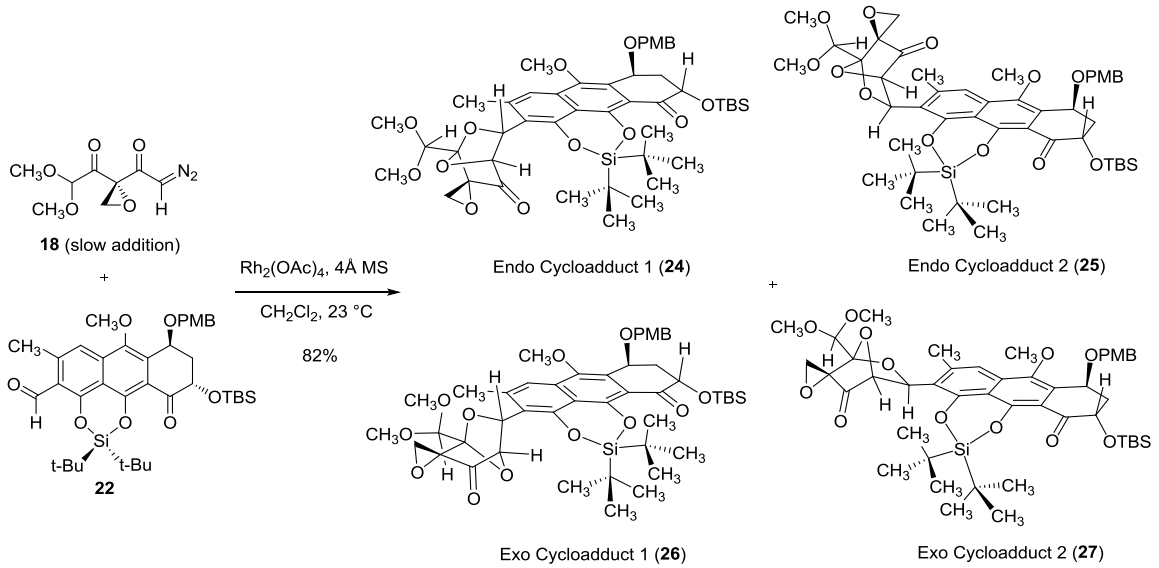



Differentially Protected Aldehyde **22**.[18]

Di-*tert*-butyldichlorosilane (2.43 mL, 11.5 mmol, 1.8 equiv) was added to a solution of 8,9-dihydroxy-7-formyl-3,4-dihydroanthracen-1-one **70** (1 equiv, see paragraph above), *N*,*N*-diisopropylethylamine (5.58 mL, 31.9 mmol, 5.0 equiv), and anhydrous 1-hydroxybenzotriazole (432 mg, 3.19 mmol, 0.5 equiv) in dimethylformamide (128 mL) at 23 °C. The reaction flask was heated in an oil bath at 55 °C. After 80 min, the heating bath was removed and the reaction flask was allowed to cool to 23 °C. The reaction mixture was partitioned between saturated aqueous sodium bicarbonate solution (300 mL) and ether (1.5 L). The layers were separated. The organic layer was washed with water (2 × 500 mL) then saturated aqueous sodium chloride solution (300 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (10% ethyl acetate–hexanes) to provide of the product **22** as a yellow foam (2.50 g, 55% over steps).[18]

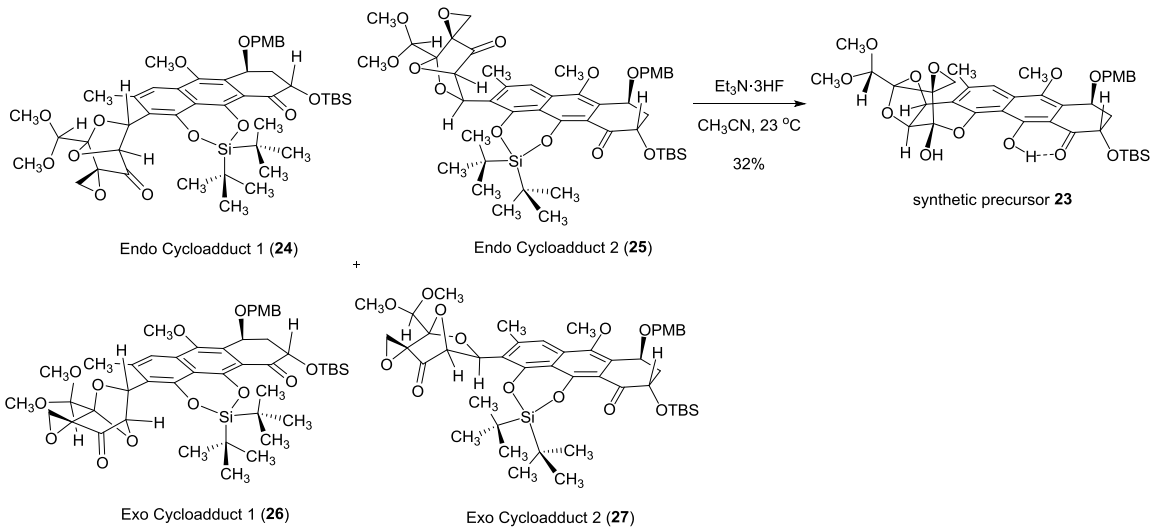
| | |
|---|---|
| **$^1$H NMR**: | 10.8 (s, 1H), 7.34 (s, 1H), 7.31 (d, 2H, *J* = 6.5 Hz), 6.88 |
| (500 MHz, $CDCl_3$) | (d, 2H, *J* = 7.0 Hz), 5.20 (dd, 1H, *J* = 3.0, 3.0 Hz), 4.88 |
| | (dd, 1H, *J* = 12.5, 5.0 Hz), 4.73 (d, 1H, *J* = 11.0 Hz), |
| | 4.63 (d, 1H, *J* = 10.5 Hz), 3.90 (s, 3H), 3.79 (s, 3H), |

| | | |
|---|---|---|
| | 2.77–2.72 (m, 1H), 2.72 (s, 3H), 2.19–2.13 (m, 1H), 1.15 (s, 9H), 1.12 (s, 9H), 0.96 (s, 9H), 0.24 (s, 3H), 0.14 (s, 3H). | |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 194.2, 190.9, 160.4, 159.4, 150.2, 146.3, 140.3, 134.0, 133.2, 130.0, 129.8, 199.4, 116.5, 115.6, 115.3, 114.0, 71.4, 71.2, 69.8, 62.7, 55.3, 36.3, 26.1, 26.0, 26.0, 22.5, 21.4, 21.0, 18.7, –4.3, –5.4. | |
| **FTIR**, $cm^{-1}$: (neat) | 2936 (s), 1786 (s), 1684 (s), 1607 (s), 1373 (s), 1247 (s), 1157 (s), 1034 (s). | |
| **HRMS**: (ESI) | Calcd for $(C_{39}H_{54}O_8Si_2+H)^+$ | 707.3430 |
| | Found | 707.3422 |
| **TLC**: (40% ethyl acetate–hexanes) | $R_f$ = 0.87 (CAM) | |




Gram-Scale Endo-Selective Carbonyl Ylide–Aldehyde Cycloaddition:

A solution of (*S*)-1-diazo-3-epoxy-5,5-dimethoxypenta-2,4-dione **18**[18] (2.27 g, 10.6 mmol, 3.0 equiv) in dichloromethane (3.9 mL) was added by a motor-driven syringe pump over 6 h to a suspension of differentially protected aldehyde **22** (2.50 g, 3.54 mmol, 1 equiv), rhodium(II) acetate (31 mg, 71 μmol, 0.02 equiv), and powdered 4-Å molecular sieves (500 mg) in dichloromethane (3.9 mL) at 23 °C. After 30 min, the reaction mixture was filtered through a short pad of silica gel (length: 10 cm; diameter: 1.5 cm), eluting with 40% ethyl acetate–hexanes to remove the rhodium(II) acetate and powdered 4-Å molecular sieves. The filtrate was concentrated. The residue was purified by flash-column chromatography (10% ethyl acetate–hexanes initially, grading to 40% ethyl acetate–hexanes) to provide, together, 2.59 g of a mixture of cycloadducts **24** (44%), **25** (37%), **26** (16%), and **27** (3%), as depicted above (82% yield).[18] In addition, 380 mg (15%) of the starting material, the differentially protected aldehyde **22**, was recovered.




Synthetic Precursor **23**.

Triethylamine–trihydrofluoride (1.42 mL, 8.70 mmol, 3.0 equiv) was added to a solution of a mixture of cycloadducts **24**, **25**, **26**, and **27** (2.59 g, 2.90 mmol, 1 equiv, as described above) in acetonitrile (58 mL) at 23 °C. After 15 min, the bright yellow solution was diluted with dichloromethane (500 mL). The diluted solution was washed sequentially with pH 7 aqueous phosphate buffer solution (100 mL) then saturated aqueous sodium chloride solution (100 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography on silica gel deactivated with triethylamine (30% ethyl acetate–hexanes initially, grading to 40% ethyl acetate–hexanes; two purifications) to provide synthetic precursor **23** as a yellow-green foam (690 mg, 32%).[18]

**$^1$H NMR**: (500 MHz, $CDCl_3$) 7.37 (s, 1H), 7.30 (d, $J$ = 9.0 Hz, 2H), 6.86 (d, $J$ = 8.5 Hz, 2H), 5.74 (d, $J$ = 3.0 Hz, 1H), 5.48 (d, $J$ = 3.5 Hz,

1H), 5.15 (dd, $J$ = 2.5, 3.0 Hz, 1 H), 4.85 (dd, $J$ = 12.0, 4.5 Hz, 1H), 4.77 (s, 1H), 4.70 (d, $J$ = 11.0 Hz, 1H), 4.60 (d, $J$ = 11.0 Hz, 1H), 3.87 (s, 3H), 3.79 (s, 3H), 3.63 (d, $J$ = 6.0 Hz, 1H), 3.61 (s, 3H), 3.61 (s, 3H), 3.07 (d, $J$ = 7.0 Hz, 1H), 2.75 (s, 3H), 2.73–2.69 (m, 1H), 2.17–2.11 (m, 1H), 1.19 (s, 9H), 1.07 (s, 9H), 0.95 (s, 9H), 0.23 (s, 3H), 0.13 (s, 3H).

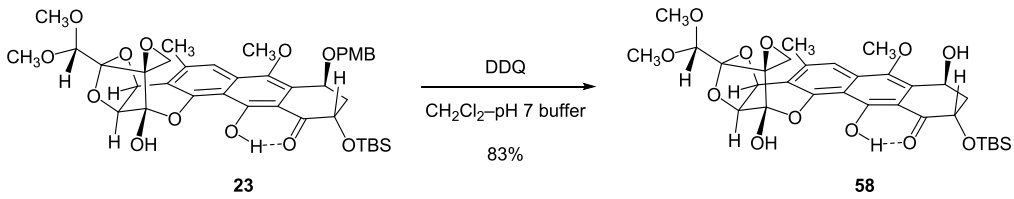
**$^{13}$C NMR**: (125 MHz, $CDCl_3$) 201.0, 194.4, 159.3, 150.1, 149.1, 146.2, 139.6, 131.2, 130.3, 129.7, 129.4, 118.2, 116.8, 115.8, 114.5, 113.8, 107.0, 101.6, 83.4, 79.6, 71.3, 71.2, 69.6, 64.3, 62.6, 56.6, 55.9, 55.3, 50.9, 36.7, 26.4, 26.1, 26.0, 23.6, 21.2, 21.2, 18.7, –4.3, –5.3.

**FTIR**, $cm^{-1}$: (neat) 2928 (s), 2857 (s), 1784 (s), 1699 (s), 1611 (s), 1371 (s), 1250 (s).

**HRMS**: (ESI)

| | |
|---|---|
| Calcd for $(C_{47}H_{64}O_{13}Si_2+H)^+$ | 893.3958 |
| Found | 893.3932 |

**TLC**: (30% ethyl acetate–hexanes) $R_f$ = 0.46 (CAM)




23 58

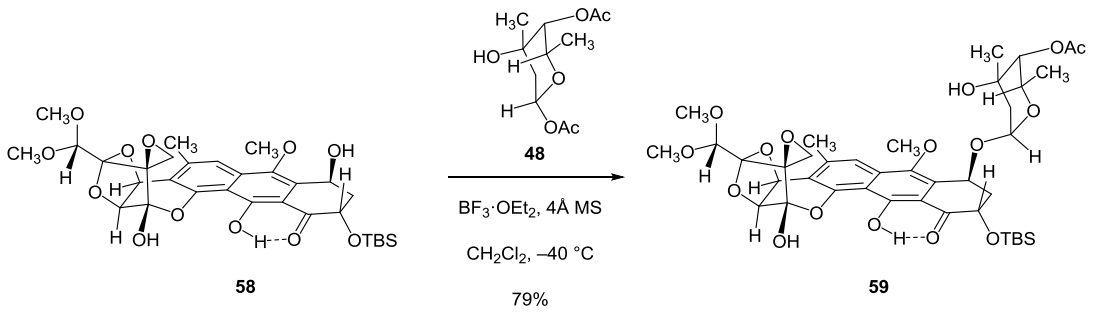
Benzylic Alcohol **58**.

2,3-Dichloro-5,6-dicyanobenzoquinone (16 mg, 70 mmol, 1.1 equiv) was added to a vigorously stirring, biphasic solution of synthetic precursor **23** (48 mg, 64 mmol, 1 equiv) in dichloromethane (1.0 mL) and pH 7 aqueous phosphate buffer solution (100 μL) at 23 °C. The reaction flask was covered with aluminum foil to exclude light. Over the course of 2 h, the reaction mixture was observed to change from myrtle green to lemon yellow. The product solution was partitioned between water (5 mL) and dichloromethane (40 mL). The layers were separated. The organic layer was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 40→90% acetonitrile in water, flow rate: 15 mL/min) to provide after concentration the pure benzylic alcohol **58** as a yellow-green powder (33.6 mg, 83%).

**$^1$H NMR**: (500 MHz, $CDCl_3$) 14.62 (s, 1H), 7.40 (s, 1H), 5.41 (t, *J* = 3.4 Hz, 1H), 5.25 (d, *J* = 4.1 Hz, 1H), 4.88 (dd, *J* = 11.7, 4.8 Hz, 1H), 4.85 (d, *J* = 4.1 Hz, 1H), 4.70 (s, 1H), 4.55 (br, 1H), 3.90 (s, 3H), 3.61 (s, 3H), 3.46 (s, 3H), 3.10 (d, *J* = 5.5 Hz, 1H), 3.02 (d, *J* = 5.5 Hz, 1H), 2.58 (s, 3H), 2.52–2.46 (m, 1H), 2.36–2.30 (m, 1H), 0.94 (s, 9H), 0.21 (s, 3H), 0.15 (s,

3H).

| | | |
|---|---|---|
| **$^{13}$C NMR**: | 202.9, 162.4, 151.5, 143.8, 142.0, 135.1, 129.5, 116.1, | |
| (125 MHz, $CDCl_3$) | 114.7, 114.7, 107.9, 103.9, 100.1, 98.5, 73.2, 69.6, 69.4, | |
| | 69.2, 62.6, 62.5, 57.0, 56.6, 50.4, 38.7, 25.8, 20.4, 18.5, –4.5, –5.3. | |
| **FTIR**, $cm^{-1}$: | 3466 (br), 2953 (w), 1622 (m), 1389 (m), 1123 (s), 1069 | |
| (neat) | (s), 945 (s), 729 (s). | |
| **HRMS**: | Calcd for $(C_{31}H_{41}O_{12}Si+H)^+$ | 633.2362 |
| (ESI) | Found | 633.2364 |
| **TLC**: | $R_f$ = 0.39 (UV, CAM) | |
| (60% ethyl acetate–hexanes) | | |

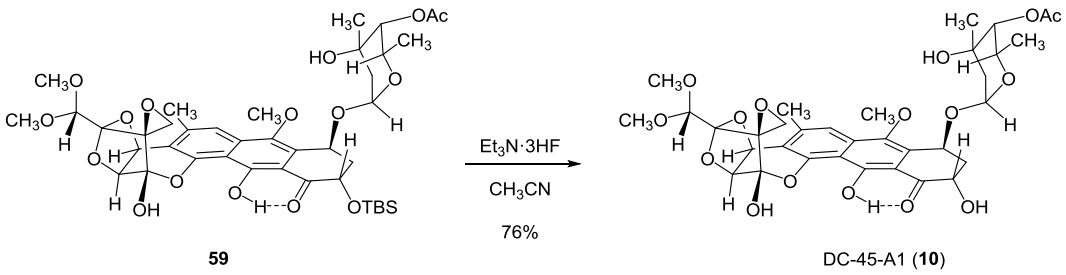



α-Glycoside **59**.

Boron trifluoride etherate (4.6 μL, 36 μmol, 1.0 equiv) was added to a suspension of benzylic alcohol **58** (23 mg, 36 μmol, 1 equiv), 1-*O*-acetyltrioxacarcinose A (**48**) (18 mg, 73 μmol, 2.0 equiv, a 1:12 mixture of α- and β-anomers, respectively), and powdered 4-Å molecular sieves (~50 mg) in dichloromethane (720 μL) at –40 °C. After 5 min, saturated aqueous sodium bicarbonate solution (1 mL) was added rapidly, and the reaction flask was allowed to warm to 23 °C. The mixture was partitioned between dichloromethane (40 mL) and saturated aqueous sodium chloride solution (5 mL). The layers were separated. The organic layer was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 40→90% acetonitrile in water, flow rate: 15 mL/min) to provide the pure α-glycoside **59** as a yellow-green powder (23.5 mg, 79%).

**$^1$H NMR**: (500 MHz, $CDCl_3$) 14.84 (s, 1H), 7.47 (s, 1H), 5.38 (d, *J* = 3.6 Hz, 1H), 5.35 (app s, 1H), 5.26 (d, *J* = 4.0 Hz, 1H), 4.84 (d, *J* = 4.0 Hz, 1H), 4.78 (dd, *J* = 12.3, 5.2 Hz, 1H), 4.75 (s, 1H), 4.71 (s, 1H), 4.52 (q, *J* = 6.6 Hz, 1H), 4.35 (s, 1H), 3.83 (s,

| | |
|---|---|
| | 3H), 3.81 (s, 1H), 3.62 (s, 3H), 3.47 (s, 3H), 3.15 (d, *J* = 5.3 Hz, 1H), 3.05 (d, *J* = 5.3 Hz, 1H), 2.60 (s, 3H), 2.58 (m, 1H), 2.35 (dt, *J* = 12.5, 2.6 Hz, 1H), 2.14 (s, 3H), 1.96 (dd, *J* = 14.6, 4.1 Hz, 1H), 1.62 (d, *J* = 14.6 Hz, 1H), 1.23 (d, *J* = 6.6 Hz, 3H), 1.08 (s, 3H), 0.95 (s, 9H), 0.24 (s, 3H), 0.16 (s, 3H). |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 202.8, 170.5, 163.2, 151.8, 144.4, 142.4, 135.2, 126.6, 116.8, 115.2, 115.1, 108.3, 104.0, 100.3, 98.6, 98.3, 74.6, 73.4, 69.8, 69.5, 69.5, 68.9, 68.4, 62.9, 62.7, 57.2, 56.8, 50.7, 38.8, 36.8, 26.0, 25.9, 21.1, 20.6, 18.6, 17.0, –4.2, –5.3. |
| **FTIR**, $cm^{-1}$: (neat) | 3516 (br), 2934 (w), 1749 (m), 1622 (m), 1391 (m), 1229 (s), 1121 (s), 995 (s), 943 (s), 868 (m), 837 (m). |
| **HRMS**: (ESI) | Calcd for $(C_{40}H_{54}O_{16}Si+Na)^+$ 841.3073<br>Found 841.3064 |
| **TLC**: (5% methanol–dichloromethane) | $R_f$ = 0.40 (UV, CAM) |



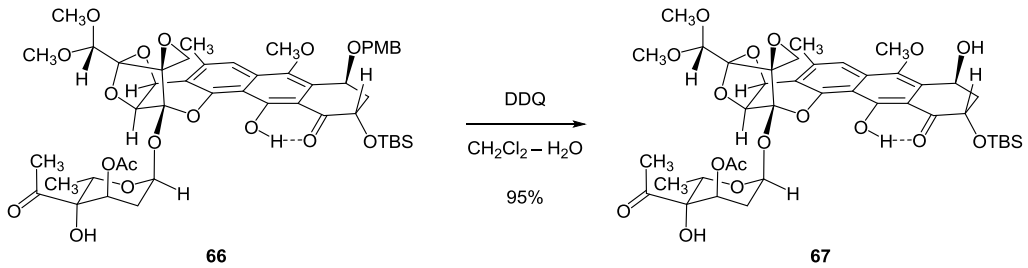

59 DC-45-A1 (**10**)

DC-45-A1 (**10**).

Triethylamine–trihydrofluoride (42 μL, 0.26 mmol, 30 equiv) was added to a solution of α-glycoside **59** (7.0 mg, 8.6 μmol, 1 equiv) in acetonitrile (290 μL) at 23 °C. The reaction flask was covered with aluminum foil to exclude light. After 15 h, the product solution was partitioned between dichloromethane (50 mL) and saturated aqueous sodium chloride solution (10 mL). The organic layer was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 20→90% acetonitrile in water, flow rate: 15 mL/min) to provide after concentration pure DC-45-A1 (**10**) as a yellow-orange powder (4.6 mg, 76%).

**$^1$H NMR**: (500 MHz, $CDCl_3$) 14.23 (s, 1H), 7.49 (s, 1H), 5.40–5.37 (m, 2H), 5.26 (d, *J* = 4.0 Hz, 1H), 4.84 (d, *J* = 3.7 Hz, 1H), 4.78–4.73 (m, 2H), 4.71 (s, 1H), 4.54 (q, *J* = 6.2 Hz, 1H), 4.43 (br, 1H), 3.84 (s, 3H), 3.62 (s, 3H), 3.58 (br, 1H), 3.47 (s, 3H), 3.16 (d, *J* = 5.1 Hz, 1H), 3.04 (d, *J* = 5.5 Hz, 1H), 2.83–2.77 (m, 1H), 2.62 (s, 3H), 2.22 (dt, *J* = 13.2, 2.6 Hz, 1H), 2.14 (s, 3H), 1.96 (dd, *J* = 14.3, 4.0 Hz, 1H), 1.61

| | | |
|---|---|---|
| | (d, $J$ = 14.3 Hz, 1H), 1.24 (d, $J$ = 6.2 Hz, 1H), 1.07 (s, 3H). | |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 202.8, 170.3, 163.1, 151.7, 144.8, 143, 135.5, 126.5, 116.8, 115.3, 114.8, 107.3, 103.9, 100.1, 98.6, 98.1, 74.4, 73.2, 69.5, 69.3, 68.8, 67.9, 67.7, 62.9, 62.7, 57.1, 56.6, 50.5, 36.7, 36.6, 25.7, 20.9, 20.5, 16.9. | |
| **FTIR**, $cm^{-1}$: (neat) | 3476 (br), 2934 (m), 1736 (s), 1622 (m), 1389 (s), 1229 (s), 1144 (s), 1082 (s), 997 (s). | |
| **HRMS**: (ESI) | Calcd for $(C_{34}H_{40}O_{16}+Na)^+$ | 727.2209 |
| | Found | 727.2200 |
| **TLC**: (5% methanol–dichloromethane) | $R_f$ = 0.30 (UV, CAM) | |



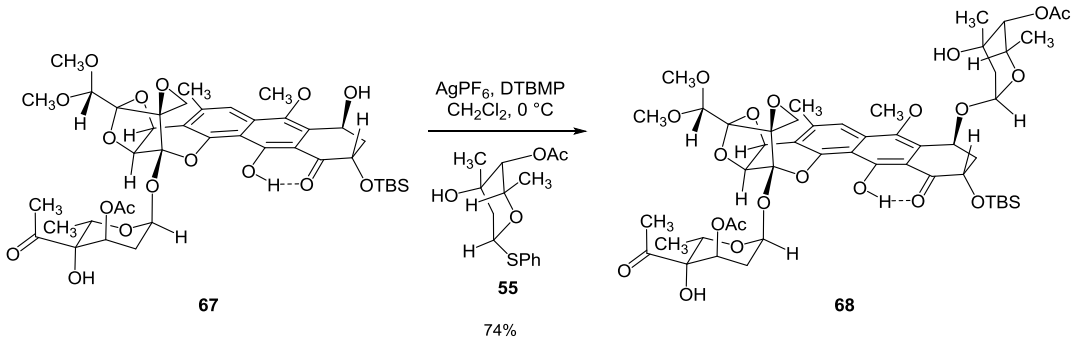

66 67

Benzylic Alcohol **67**.

To a vigorously stirring, biphasic solution of α-glycoside **66** (120 mg, 0.124 mmol, 1 equiv) in dichloromethane (1.9 mL) and pH 7 phosphate buffer (380 μL) at 23 °C was added 2,3-dichloro-5,6-dicyanobenzoquinone (33.8 mg, 0.149 mmol, 1.1 equiv). The reaction flask was covered with aluminum foil to exclude light. Over the course of 2 h, the color of the reaction mixture changed from myrtle green to yellow. The product solution was partitioned between water (10 mL) and dichloromethane (60 mL). The layers were separated. The aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 40→90% acetonitrile in water, flow rate: 15 mL/min) to provide after concentration the pure benzylic alcohol **67** (100 mg, 95%, in fractions eluting at 32.6–36.5 min) as a bright yellow solid.

**$^1$H NMR**: (500 MHz, $CDCl_3$) 14.40 (s, OH). 7.43 (s, 1 H), 5.82 (t, *J* = 2.4 Hz, 1H), 5.43 (app d, *J* = 1.8 Hz, 1H), 5.30 (d, *J* = 4.2 Hz, 1H), 5.30 (s, 1H), 5.19 (d, *J* = 3.6 Hz, 1H), 5.01 (q, *J* = 6.6 Hz, 1H), 4.90 (dd, *J* = 12.0, 4.8 Hz, 1H), 4.75 (app t, *J* = 3.6

| | | |
|---|---|---|
| | Hz, 1H), 4.74 (s, 1H), 3.90 (s, 3H), 3.89 (s, OH), 3.63 (s, 3H), 3.47 (s, 3H), 2.83 (d, *J* = 6.0 Hz, 1H), 2.74 (d, *J* = 5.4 Hz, 1H), 2.60 (s, 3H), 2.51–2.48 (m, 1H), 2.42 (br s, 1H), 2.36 (s, 3H), 2.35–2.30 (m, 2H), 2.23 (s, 3H), 1.08 (d, *J* = 6.6 Hz, 3H), 0.95 (s, 9H), 0.23 (s, 3H), 0.16 (s, 3H). | |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 208.7, 202.9, 170.3, 162.6, 151.8, 143.7, 142.1, 135.2, 129.6, 116.0, 114.8, 114.3, 107.9, 104.5, 101.6, 99.4, 92.4, 78.1, 72.10, 70.6, 69.3, 69.1, 68.2, 64.4, 62.7, 62.6, 56.6, 55.8, 47.4, 38.8, 28.9, 27.0, 25.8, 21.3, 20.4, 18.5, 14.5, –4.5, –5.4. | |
| **FTIR**, $cm^{-1}$: (neat) | 3475 (m), 2934 (s), 2857 (m), 1738 (s), 1721 (s), 1620 (s), 1570 (m), 1445 (m), 1389 (s), 1240 (s), 1082 (s). | |
| **HRMS**: (ESI) | Calcd for $(C_{41}H_{54}O_{17}Si+Na)^+$ | 869.3023 |
| | Found | 869.2793 |
| **TLC**: (60% ethyl acetate–hexanes) | $R_f$ = 0.31 (UV, CAM) | |
| **$[\alpha]^{23}_D$**: | +2.9° (*c* 0.614, $CHCl_3$) | |




3"-*O*-Acetyl-2-*O*-(*tert*-butyldimethylsilyl)-trioxacarcin A (**68**).

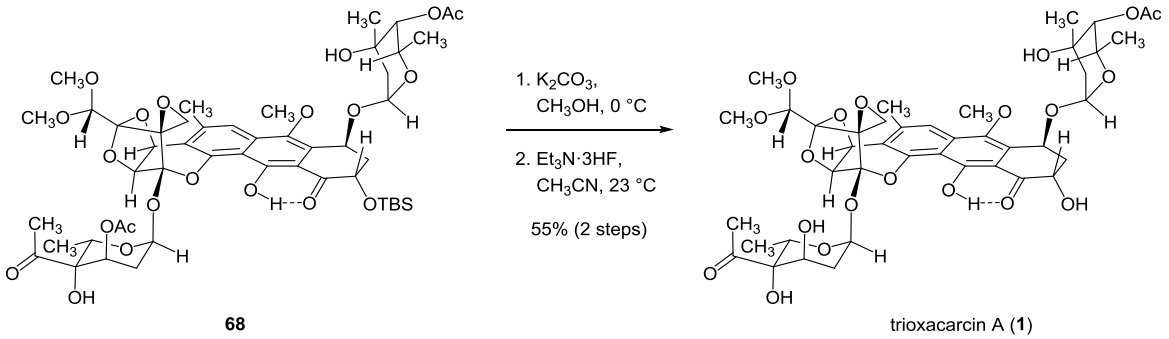
Silver hexafluorophosphate (116 mg, 0.460 mmol, 6.0 equiv) was added to a suspension of benzylic alcohol **67** (65.0 mg, 0.077 mmol, 1 equiv), 1-phenylthiotrioxacarcinoside A **55** (68 mg, 0.23 mmol, 3.0 equiv, a ~1:2.4 mixture of α and β anomers, respectively), 2,6-di-*tert*-butyl-4-methylpyridine (126 mg, 0.614 mmol, 8.0 equiv), and powdered 4-Å molecular sieves (~100 mg) in dichloromethane (3.8 mL) at 0 °C. After 1 h, pyridine (310 μL, 3.84 mmol, 50 equiv) was added, and the reaction flask was allowed to warm to 23 °C. After 30 min, the product mixture was filtered through a short pad of Celite, washing with dichloromethane (100 mL). The filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 40→100% acetonitrile in water, flow rate: 15 mL/min) to provide after concentration pure 3"-*O*-acetyl-2-*O*-(*tert*-butyldimethylsilyl)-trioxacarcin A (**68**) as a yellow-green foam (58.3 mg, 74%).

**$^1$H NMR**: (500 MHz, $CDCl_3$) 14.59 (s, 1H), 7.46 (s, 1H), 5.81 (t, *J* = 2.7 Hz, 1H), 5.37–5.35 (m, 2H), 5.31 (d, *J* = 4.4 Hz, 1H), 5.19 (d, *J* = 4.0 Hz, 1H), 5.01 (q, *J* = 6.2 Hz, 1H), 4.78 (dd, *J* = 12.5,

| | |
|---|---|
| | 5.1 Hz, 1H), 4.76–4.74 (m, 3H), 4.52 (q, 6.6 Hz, 1H), 3.89 (br, 1H), 3.82 (s, 3H), 3.63 (s, 3H), 3.47 (s, 3H), 2.84 (d, $J$ = 5.9 Hz, 1H), 2.76 (d, $J$ = 5.9 Hz, 1H), 2.59 (s, 3H), 2.58–2.54 (m, 1H), 2.36 (s, 3H), 2.35–2.32 (m, 3H), 2.23 (s, 3H), 2.14 (s, 3H), 1.95 (dd, $J$ = 14.7, 4.0 Hz, 1H), 1.63 (d, $J$ = 14.7 Hz, 1H), 1.23 (d, $J$ = 6.6 Hz, 3H), 1.09–1.07 (m, 3H), 1.08 (s, 3H), 0.96 (s, 9H), 0.25 (s, 3H), 0.16 (s, 3H). |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 208.6, 202.6, 170.3, 170.2, 163.1, 151.8, 144.1, 142.2, 134.8, 126.4, 116.4, 114.9, 114.6, 108.1, 104.4, 101.5, 99.3, 98.0, 92.3, 78.0, 74.3, 71.9, 70.5, 69.2, 68.9, 68.7, 68.1, 68.0, 64.4, 62.6, 62.5, 56.4, 55.8, 47.3, 38.7, 36.5, 29.6, 28.8, 26.8, 25.7, 21.2, 20.8, 20.2, 18.3, 16.8, 14.4, –4.4, –5.6. |
| **FTIR**, $cm^{-1}$: (neat) | 3482 (br), 2934 (w), 1740 (m), 1620 (m), 1389 (m), 1227 (s), 1117 (s), 1082 (s), 995 (s), 943 (s). |
| **HRMS**: (ESI) | Calcd for $(C_{50}H_{68}O_{21}Si+H)^+$ 1033.4095<br>Found 1033.4008 |
| **TLC**: | $R_f$ = 0.39 (UV, CAM) |

(60% ethyl acetate–hexanes)

$[\alpha]^{23}_{D}$: –33.5° (*c* 0.71, $CH_2Cl_2$)




Trioxacarcin A (**1**).

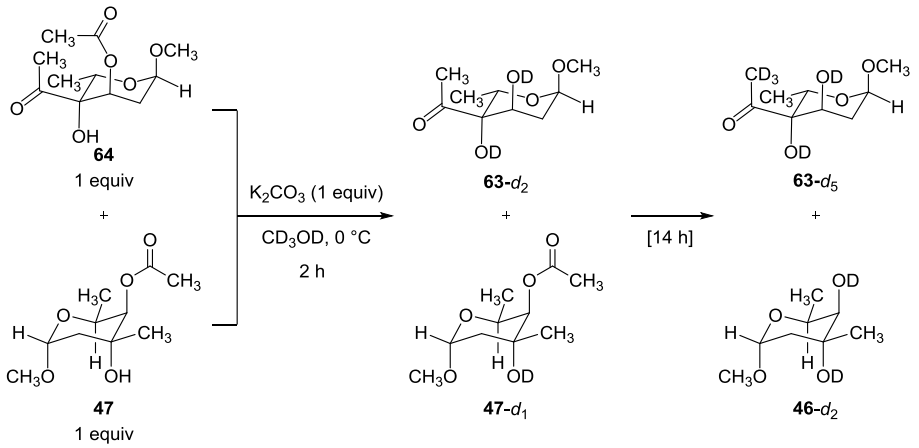
Potassium carbonate (13 mg, 95 μmol, 2.0 equiv) was added to a solution of 3"-*O*-acetyl-2-*O*-(*tert*-butyldimethylsilyl)-trioxacarcin A (**68**) (49 mg, 47 μmol, 1 equiv) in methanol (9.5 mL) at 0 °C. After 60 min, ammonium chloride (10 mg, 0.19 mmol, 4.0 equiv) was added. After 1 min, the mixture was filtered through a short pad of Celite, washing with methanol (50 mL). The filtrate was concentrated. Analysis of the residue by $^1$H NMR spectroscopy (500 MHz, $CDCl_3$) established that the 3"-*O*-acetyl protecting group (δ 2.22, (s, 3H)) had been cleaved and that the 4'-*O*-acetyl group was retained (δ 2.13 (s, 3H)). The residue (1 equiv, see above) was dissolved in acetonitrile (1.6 mL), and triethylamine–trihydrofluoride (232 μL, 1.4 mmol, 30 equiv) was added at 23 °C. The reaction flask was covered with aluminum foil to exclude light. After 14 h, saturated aqueous sodium bicarbonate solution (10 mL) was added. The mixture was extracted with dichloromethane (2 × 50 mL). The organic layers were combined. The combined solution was dried over sodium sulfate. The dried solution was filtered and filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 20→80% acetonitrile in water, flow rate: 15 mL/min) to provide after concentration pure trioxacarcin A (**1**) as an orange-red powder (23 mg, 55% yield over two steps).

| | |
|---|---|
| **$^{1}$H NMR**: (500 MHz, $CDCl_3$) | 14.05 (s, 1H), 7.51 (s, 1H), 5.85 (d, *J* = 2.6 Hz, 1H), 5.39 (m, 1H), 5.37 (d, *J* = 4.0 Hz, 1H), 5.24 (d, *J* = 4.0 Hz, 1H), 5.02 (q, *J* = 6.6 Hz, 1H), 4.78 (m, 1H), 4.77 (s, 1H), 4.75 (app s), 4.54 (q, *J* = 6.6 Hz, 1H), 4.24 (d, *J* = 9.2 Hz, 1H), 4.16 (br, 1H), 3.84 (s, 3H), 3.72 (br d, *J* = 8.8 Hz, 1H), 3.63 (s, 3H), 3.56 (s, 1H), 3.49 (s, 3H), 2.99 (d, *J* = 5.9 Hz, 1H), 2.91 (d, *J* = 5.5 Hz, 1H), 2.81 (m, 1H), 2.62 (s, 3H), 2.50 (s, 3H), 2.45 (dt, *J* = 14.3, 3.3 Hz, 1H), 2.21 (dt, *J* = 13.2, 2.2 Hz, 1H), 2.14 (s, 3H), 2.11 (m, 1H), 1.97 (dd, *J* = 14.7, 4.0 Hz, 1H), 1.62 (d, *J* = 14.7 Hz, 1H), 1.24 (d, *J* = 6.6 Hz, 3H), 1.10 (d, *J* = 6.2 Hz, 3H), 1.07 (s, 3H). |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 14.5, 16.8, 20.3, 20.9, 25.7, 27.8, 31.5, 36.6, 48.1, 56.2, 56.8, 62.7, 62.9, 63.8, 67.4, 67.9, 68.3, 68.8, 69.1, 70.2, 71.4, 74.4, 79.5, 94.8, 97.9, 99.7, 101.5, 104.6, 107.4, 114.8, 116.9, 126.6, 135.4, 142.8, 144.8, 151.7, 163.1, 170.3, 202.9, 210.3. |
| **FTIR**, $cm^{-1}$: (neat) | 3514 (br), 2976 (w), 2940 (w), 1748 (m), 1620 (m), 1387 (m), 1225 (s), 1086 (s), 997 (s). |

| | | |
|---|---|---|
| **HRMS**: | Calcd for $(C_{42}H_{52}O_{20}–H)^-$ | 875.2979 |
| (ESI) | Found | 875.2961 |

**TLC**: $R_f$ = 0.25 (UV, CAM)

(80% ethyl acetate–hexanes)




Competition Experiment: Diols **63** and **46**.

Potassium carbonate (5.7 mg, 0.040 mol, 1.0 equiv) was added to a solution of methyl α-3-*O*-acetyltrioxacarcinoside B (**64**)[65] (9.0 mg, 0.040 mmol, 1 equiv) and methyl α-trioxacarcinoside A (**47**) (10.2 mg, 0.040 mmol, 1 equiv) in methanol-$d_4$ (3 mL) at 0 °C. After 2 h, analysis of the solution by $^1$H NMR spectroscopy (500 MHz, $CD_3OD$) established that the 3-*O*-acetyl protecting group of **64** had been cleaved to afford methyl α-trioxacarcinoside B (diol **63**-$d_2$) and that the 4-*O*-acetyl group of **47** was retained. After 16 h, $^1$H NMR analysis showed that the 4-*O*-acetyl group of **47** had been cleaved to give diol **46**-$d_2$ as the major product; additionally, complete proton-deuterium exchange of the methyl ketone of **63**-$d_2$ had occurred to give **63**-$d_5$.

# Chapter 4

# Preparation of Fully Synthetic Trioxacarcin Analogs

## Introduction

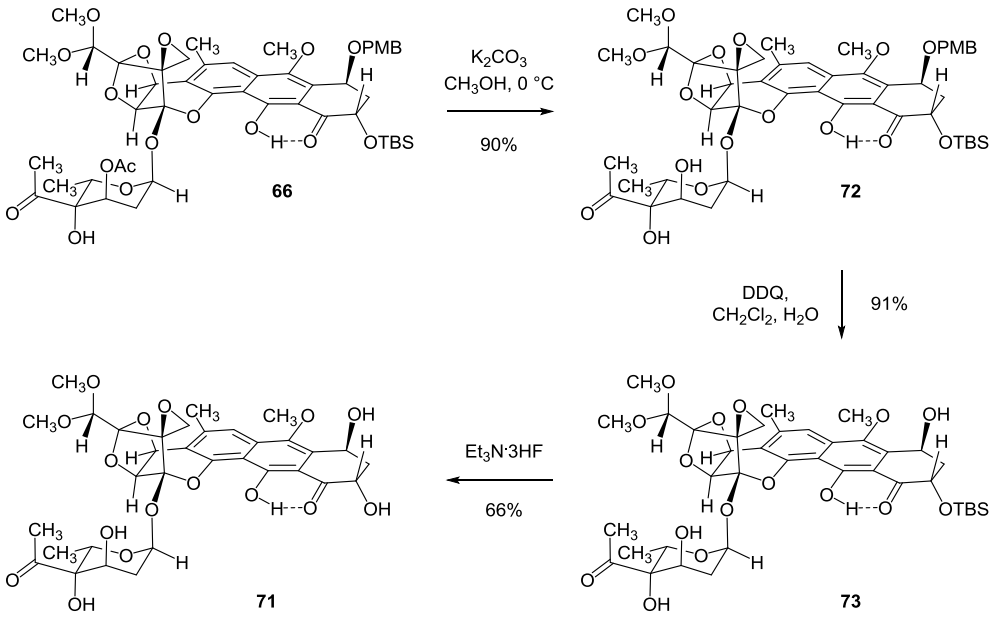
In the previous chapter, I presented a synthetic route to trioxacarcin A (**1**) that proceeded by the efficient assembly of five components of similar synthetic complexity: the epoxy diazo diketone **18**, the cyanophthalide **19**, the cyclohexenone **20**, the trioxacarcinose A donor **55**, and the trioxacarcinose B donor **65**. We had envisioned that this component-based assembly process would allow the relatively rapid introduction of deep-seated structural modifications by modification of the components. In this chapter I discuss the results of efforts related to the synthesis of novel trioxacarcin analogs, using component modification as our primary strategy. The compounds detailed herein represent a substantial expansion of the pool of known trioxacarcins and the realization of a key aspect of our synthetic approach to the trioxacarcin class.

In the first section I briefly describe the synthesis of a novel trioxacarcin monoglycoside analog **71**, formally 4-deglyco-trioxacarcin A (**1**). Three subsequent sections present efforts in analog synthesis through modification, in turn, of enone component **20**, cyanophthalide **19**, and glycosidic components **55** and **65**. The final section discusses early efforts in trioxacarcin analog synthesis not by component modification but by end-stage transformations of fully functionalized trioxacarcins.

## Synthesis of a Novel Trioxacarcin Monoglycoside

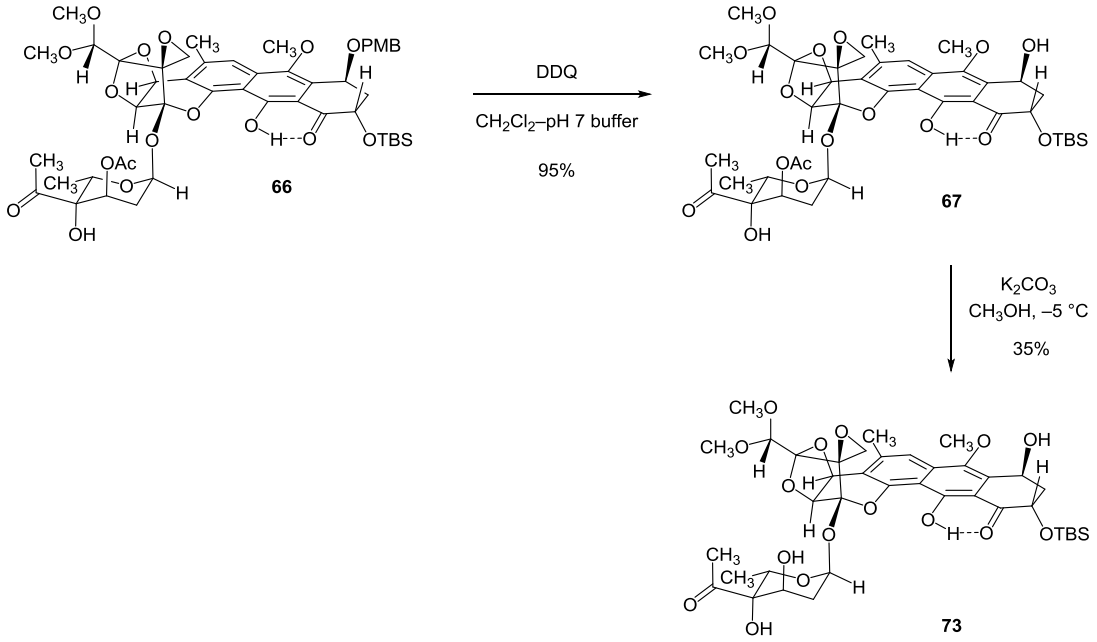
While a wide variety of glycosylated trioxacarcins are known (see Figure 1.1 and Figure 1.2), the monoglycoside **71**, which contains only a trioxacarcinose B residue, has not been identified as a natural product. From our glycosylation studies, the

monoglycoside intermediate **66** was available in one step from differentially protected aglycon **23** (see Scheme 3.6). This intermediate **66** represents a protected form of the monoglycoside **71** and was converted to this analog by a sequence of three deprotection steps. First, cleavage of the acetate ester within the trioxacarcinose B residue by treatment with potassium carbonate in methanol at 0 °C proceeded efficiently to afford alcohol **72** in 90% yield (Scheme 4.1). Removal of the PMB ether protective group (DDQ, 91% yield) afforded benzylic alcohol **73**, and subsequent cleavage of the TBS ether group within this product ($Et_3N{\cdot}3HF$, 66% yield) provided the novel trioxacarcinose B monoglycoside **71** as a yellow-green foam after careful purification by RP-HPLC.




**Scheme 4.1.** Synthesis of Trioxacarcinose B Monoglycoside **71**.

Interestingly, when we performed the deprotection steps in a different order, the yield of the deacetylation reaction suffered. Removal of the PMB ether group within intermediate **66**, an operation used in the synthesis of trioxacarcin A (**1**) (see Scheme 3.7, DDQ, 95% yield) provided benzylic alcohol **67**, and deacetylation of this product ($K_2CO_3$, $CH_3OH$, –5 °C) proceeded in only 35% yield (Scheme 4.2). This observation suggested that the sensitivity of trioxacarcins to basic conditions may depend strongly on the nature of the substitution of the cyclohexenone ring.




**Scheme 4.2.** Deacetylation of Benzylic Alcohol **67**.

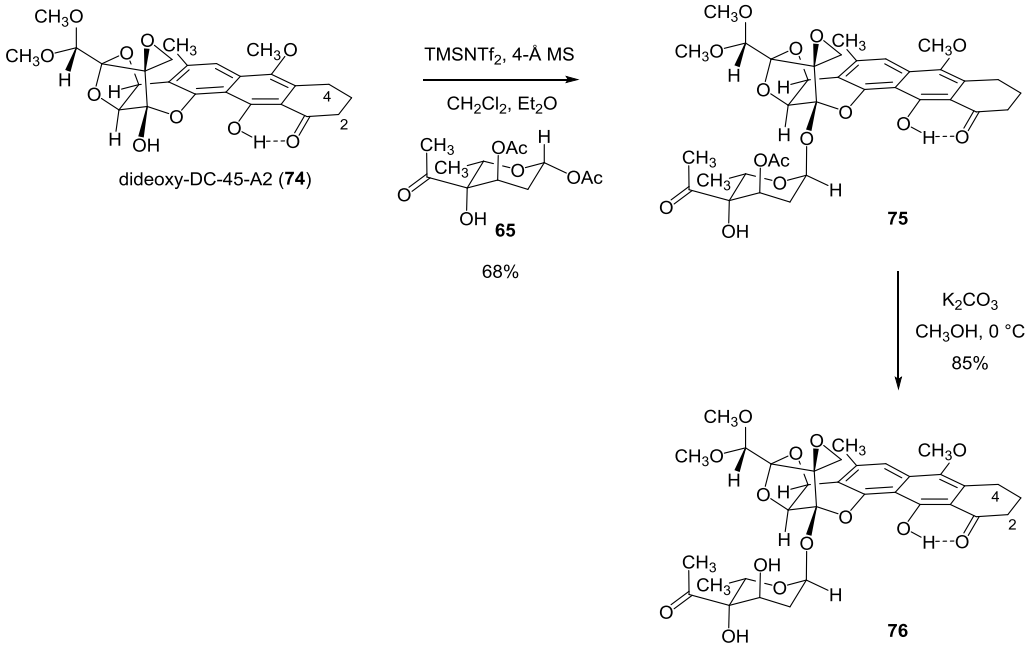
## Synthesis of Deoxygenated Trioxacarcins

Having established a modular sequence for the assembly of trioxacarcin A (**1**) from five components of similar synthetic complexity (see Chapter 3), we next sought to

demonstrate the versatility of the sequence for the synthesis of analogs by component variation. As an important first step toward this end, graduate students Jakub Švenda and Nicholas Hill reported the preparation of dideoxy-DC-45-A2 (**74**), so named because it lacks the hydroxyl groups present at positions C2 and C4 within DC-45-A2 (**11**). This novel trioxacarcin was prepared without variation of the synthetic route, save for the use of 2-cyclohexen-1-one as starting material in place of enone component **20**. The use of 2-cyclohexen-1-one, a commercially available and achiral material, simplified the synthetic sequence greatly relative to the use of the chiral enone component **20**, which required 10 steps for its preparation from L-malic acid.[18]

Having developed methodology for the selective coupling of trioxacarcinose A (**2**) and trioxacarcinose B (**3**) donors to trioxacarcin aglycon substrates, we sought to develop further the idea of analog synthesis by component variation through the preparation of glycosylated, deoxygenated trioxacarcins. First, we prepared glycosylated analog **76** by a simple two-step sequence of glycosylation followed by deprotection (Scheme 4.3). Using the glycosylation protocol developed by postdoctoral researcher Thomas Magauer, with *N*-trimethylsilyl-bis(trifluoromethanesulfonyl)imide as the Lewis-acidic promoter, 1,3-di-*O*-acetyl trioxacarcinose B (**65**) was coupled to dideoxy-DC-45-A2 (**74**) to provide the α-glycoside **75** in 68% yield; the β-glycoside was not observed. Methanolysis of the acetate ester protective group within this product, using potassium carbonate as base, provided novel deoxygenated trioxacarcin analog **76**, which in comparison to monoglycoside analog **71** above lacks hydroxyl groups at positions C2 and C4. It is noteworthy that methanolysis of glycoside **75** with potassium carbonate proceeded in a far higher yield than the corresponding methanolysis of bisglycoside **68**

(see Scheme 3.10). This observation served as an indication that dideoxy trioxacarcins such as **76** may be more stable to basic conditions than the analogous structures containing hydroxyl groups or glycosides at positions C2 and C4.




**Scheme 4.3.** Synthesis of Glycosylated 2,4-Dideoxy Trioxacarcin Analog **76**.

Having prepared a glycosylated 2,4-dideoxy trioxacarcin analog (**76**), we sought to prepare so-called 2-deoxy trioxacarcins, differing from natural trioxacarcins only by the absence of the C2 hydroxyl group. These studies provided an opportunity to study the effect of the absence of the C2 hydroxyl group on the antiproliferative activity of the resulting analogs. Furthermore, since the C4 hydroxyl group present within naturally occurring trioxacarcins would be retained within this series, we retained the opportunity to couple glycosyl residues such as trioxacarcinose A (**2**) to the 4-hydroxyl position.

For the preparation of 2-deoxy trioxacarcins we initially made use of protected aglycon **78**, which lacks the *tert*-butyldimethylsilyloxy group present in differentially protected synthetic precursor **23** but is otherwise identical. The preparation of aglycon **78** in turn required multigram quantities of the modified enone component (*S*)-4-((4-methoxybenzyl)oxy)cyclohex-2-en-1-one (**77**) (Figure 4.1). Danishefsky and coworkers have reported a route to this compound proceeding in 7 steps from D-quinic acid; in their report, the authors describe the preparation of 3.80 g of enone **77**.[68] Graduate student Nicholas Hill executed this 7-step route on a multigram scale using 20 g of D-quinic acid as starting material and obtained 1.9 g of enone **77** in 8% overall yield, providing material to supply initial synthetic studies of 2-deoxy trioxacarcins.

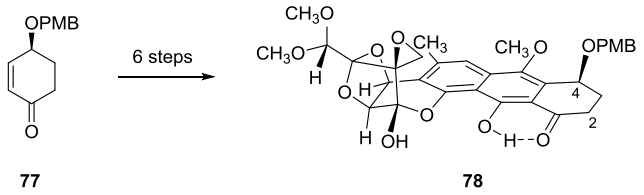
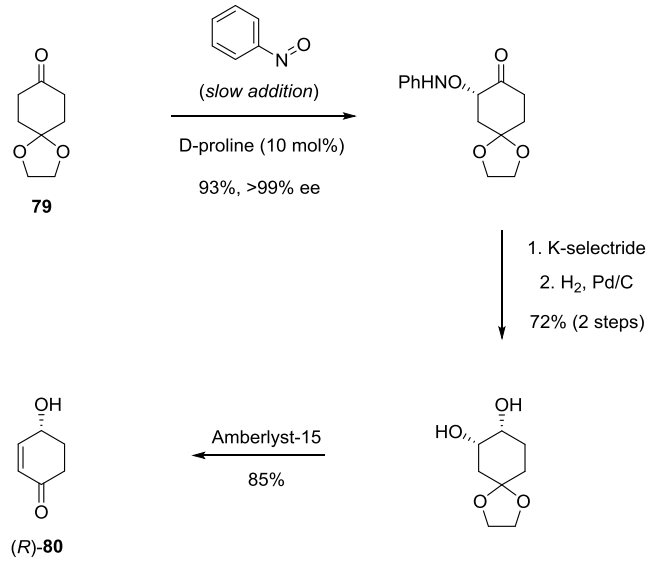



**Figure 4.1.** Preparation of Synthetic Precursor **78** for the Synthesis of 2-Deoxy Trioxacarcins Required Enone **77** as Starting Material.

We considered other routes to enone **77** that would be suitable for delivering multigram quantities of this material and took note of a report by Matsuzawa and coworkers of the enantioselective preparation of (*R*)-4-hydroxy-2-cyclohexen-1-one (*R*)-**80** in the context of a synthetic route to (+)-panepophenanthrin.[69] The synthesis of (*R*)-**80**

[68] Audia, J. E.; Boisvert, L.; Patten, A. D.; Villalobos, A.; Danishefsky, S. J. *J. Org. Chem.* **1989**, *54*, 3738–3740.
[69] Matsuzawa, M.; Kakeya, H.; Yamaguchi, J.; Shoji, M.; Onose, R.; Osada, H.; Hayashi, Y. *Chem.–Asian J.* **2006**, *1*, 845–851.

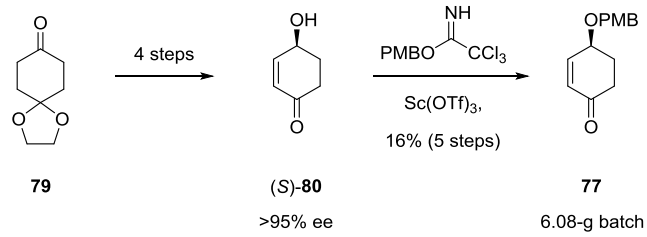
proceeded in four steps from cyclohexanedione monoethylene ketal (**79**) and used a catalytic, asymmetric, D-proline-mediated aminoxylation reaction as the key step(Scheme 4.4). The authors reported the preparation of 64.6 mg of (*R*)-4-hydroxy-2-cyclohexen-1-one (**80**) in 57% overall yield using this four-step sequence.




**Scheme 4.4.** Synthesis of (*R*)-4-Hydroxy-2-Cyclohexen-1-One (*R*)-**80** as Reported by Matsuzawa et al.[69]

I carried out the route reported by Matsuzawa and coworkers on a multigram scale with 26.35 g of cyclohexanedione monoethylene ketal (**79**) as starting material, using L-proline as catalyst in the enantioselective aminoxylation reaction in order to prepare 4-hydroxy-2-cyclohexen-1-one of the opposite enantiomeric series (Scheme 4.5). After protection of the hydroxyl function of (*S*)-4-hydroxy-2-cyclohexen-1-one ((*S*)-**80**)

prepared in this manner[70] as the *p*-methoxybenzyl ether, I obtained 6.08 g of enone building block **77** in 16% overall yield over the 5-step sequence.

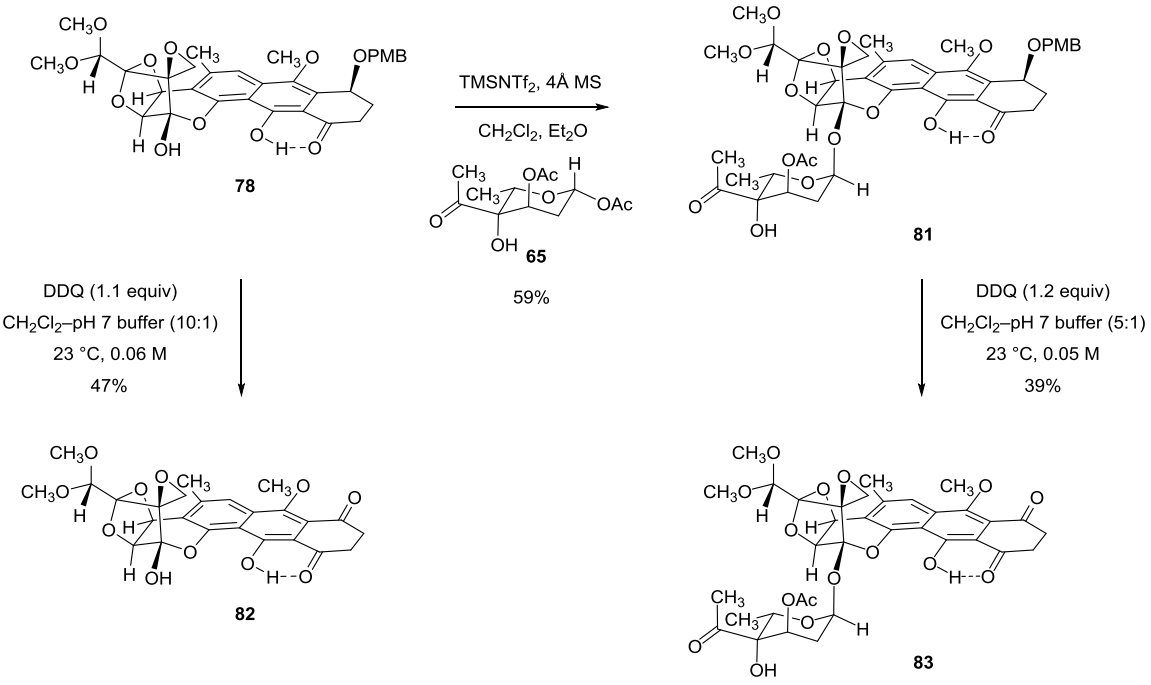



**Scheme 4.5.** Synthesis of Enone Component **77** Using the Route Reported by Matsuzawa et al.[69]

The above route to enone building block **77** provided sufficient material for the preparation of various 2-deoxy trioxacarcin analogs. As an entry point to this analog series, aglycon **78** (Scheme 4.6) was assembled from the epoxy diazo diketone **18**, cyanophthalide **19**, and enone **77** by an analogous sequence to that used for the synthesis of synthetic precursor **23** (see Scheme 1.3). Glycosylation with trioxacarcinose B donor **65** using our established protocol, with *N*-trimethylsilyl-bis(trifluoromethanesulfonyl)imide as the Lewis-acidic promoter, provided the α-glycoside **81** in 59% yield (Scheme 4.6). However, attempted cleavage of the *p*-methoxybenzyl ether within either aglycon **78** or monoglycoside **81** with DDQ in a mixture of dichloromethane and pH 7 aqueous phosphate buffer furnished 1,4-diketone products **82** and **83**, respectively, rather than the desired benzylic alcohols. This stands in contrast to the deprotection reaction of monoglycoside **66**—which differs from **81** only in the presence of a 2-*tert*-butyldimethylsilyloxy substituent—in which the diketone product

[70] (*S*)-**80** prepared in this manner was found to be of >95% ee by analysis of the $^{1}H$ and $^{19}F$ NMR spectra of the corresponding Mosher ester.

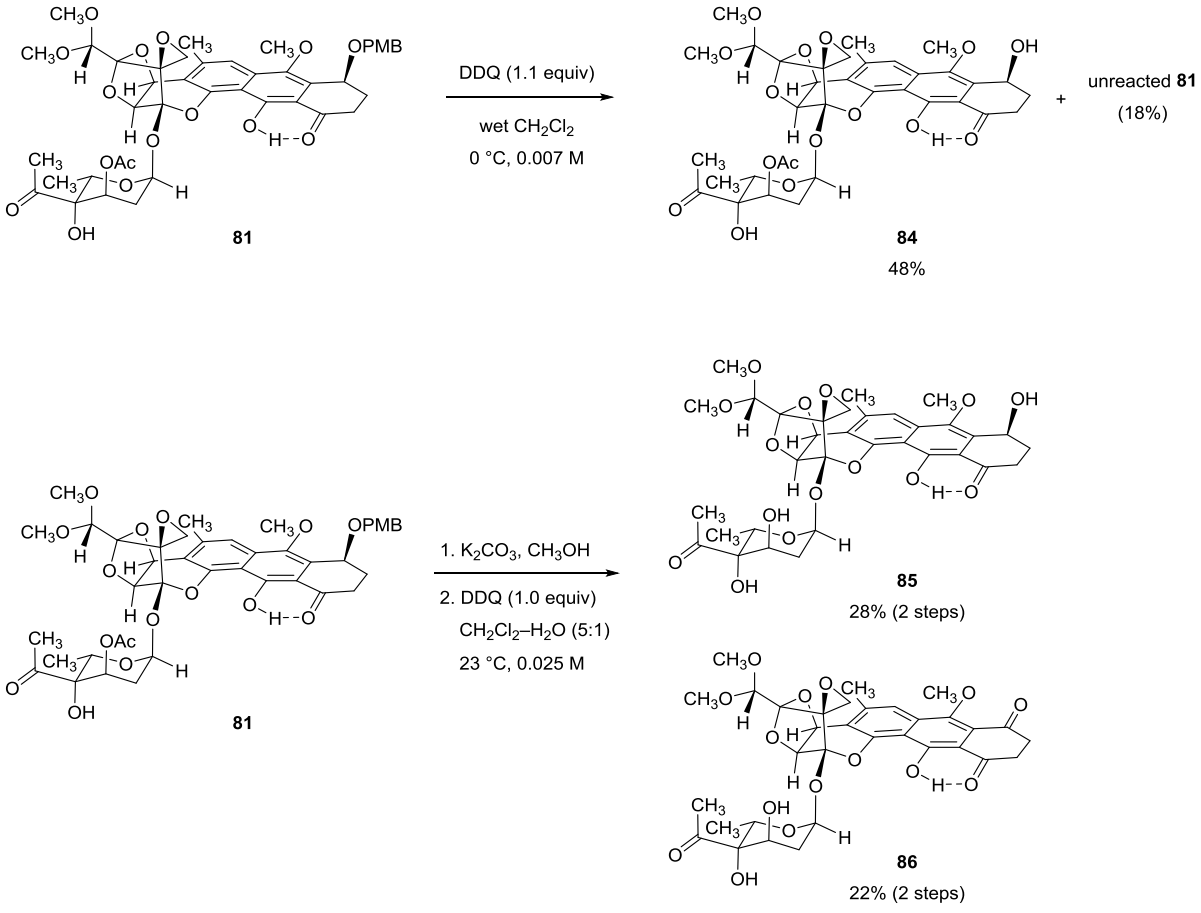
was not observed and the benzylic alcohol **67** was isolated in 95% yield (see Scheme 3.7).




**Scheme 4.6.** Synthesis of Glycoside **81** from Synthetic Precursor **78**, and Formation of 1,4-Diketone Products **82** and **83**.

Evidence suggests that the corresponding benzylic alcohol is an intermediate in the oxidation process which forms the diketone functionality; indeed, a separate experiment run in dichloromethane–water at 0 °C under more dilute conditions (0.007 M in substrate) provided benzylic alcohol **84** from substrate **81** in 48% yield and, separately, unreacted *p*-methoxybenzyl ether starting material **81** in 18% yield (Scheme 4.7). Likewise, in an experiment conducted by postdoctoral researcher Thomas Magauer, methanolysis of **81** with potassium carbonate as base followed by treatment of the product with DDQ in dichloromethane–water at 23 °C (0.025 M in substrate) provided a

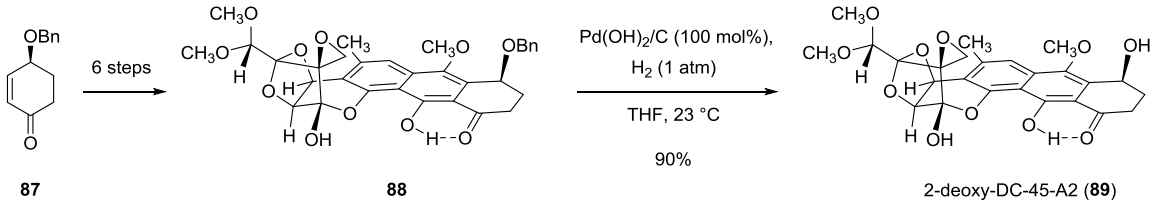
separable mixture of benzylic alcohol **85** (28% yield) and diketone **86** (22% yield). We were unable to suppress completely the formation of these diketones while also achieving high conversion of the *p*-methoxybenzyl ether substrates. While DDQ-promoted deprotection of aglycon **78** and the corresponding monoglycoside derivatives failed to provide an efficient approach to 2-deoxy trioxacarcin analogs bearing a C4 benzylic alcohol, the C4-oxo compounds **82**, **83**, and **86** represent a novel structural motif within the trioxacarcin class.




**Scheme 4.7.** Formation of Benzylic Alcohols **84** and **85** from PMB-Protected Glycoside **81**.

Having observed a benzylic oxidation side reaction during attempted cleavage of *p*-methoxybenzyl ether substrates such as **78** and **81**, we considered alternative deprotection conditions or protective groups which would allow the benzylic alcohol at position C4 to be revealed late in our routes to 2-deoxy trioxacarcins. Given that these substrates appeared to be particularly vulnerable to oxidative reaction conditions such as treatment with DDQ, a benzyl ether seemed a promising candidate for protection of the 4-hydroxyl function, as we expected deprotection under orthogonal conditions of reductive hydrogenolysis to be facile.

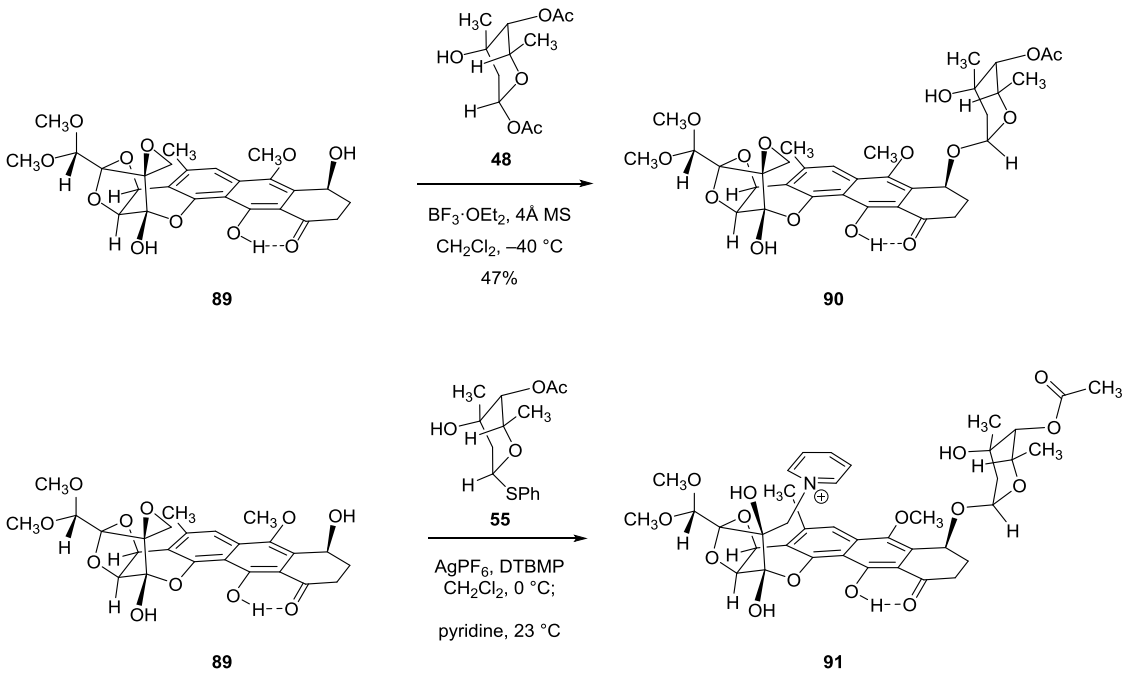
Benzylation of (*S*)-4-hydroxy-2-cyclohexen-1-one ((*S*)-**80**) (silver(I) oxide, benzyl bromide, dichloromethane, 23 °C, 52% yield) provided the new enone component **87**. We prepared benzyl-protected aglycon **88** from epoxy diazo diketone **18**, cyanophthalide **19**, and enone **87** by an analogous sequence to that employed for the synthesis of synthetic precursor **23** (see Scheme 1.3). Deprotection of aglycon **88** was facile and high-yielding. Stirring a suspension of aglycon **88** and palladium hydroxide on carbon in tetrahydrofuran under a hydrogen atmosphere provided 2-deoxy-DC-45-A2 (**89**) in 90% yield (Scheme 4.8).




**87** **88** 2-deoxy-DC-45-A2 (**89**)

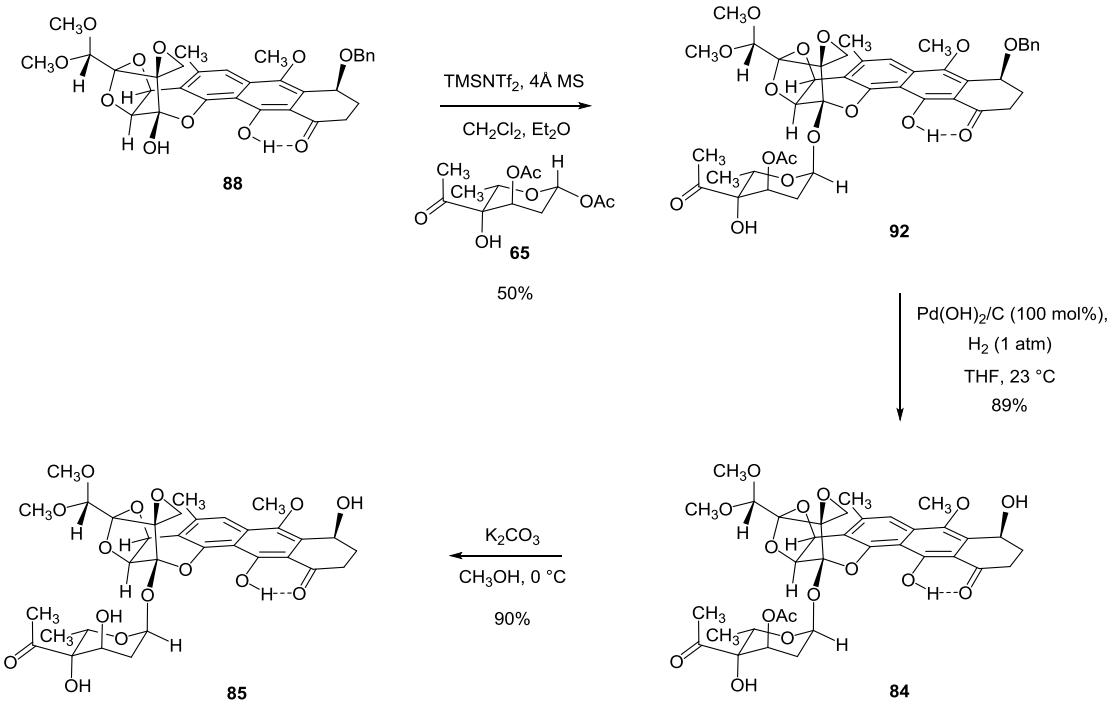
**Scheme 4.8.** Synthesis of 2-Deoxy-DC-45-A2 (**89**) by Hydrogenolysis of Benzyl Ether **88**.

We next sought to prepare the 2-deoxy analogs of many of the fully synthetic trioxacarcins we had prepared thus far. Glycosylation of 2-deoxy-DC-45-A2 (**89**) with 1-*O*-acetyl trioxacarcinose A (**48**) using boron trifluoride etherate as Lewis-acidic promoter afforded 2-deoxy-DC-45-A1 (**90**) in a single step (47%, Scheme 4.9). Interestingly, use of the glycosylation protocol that was successfully employed to prepare bisglycoside **68** during our synthesis of trioxacarcin A, for which excess pyridine was added after the reaction is complete (see Scheme 3.9), resulted in formation of alkylated pyridinium adduct **91** (Scheme 4.9). This adduct was identified based on analysis of its $^{1}$H NMR spectrum and by observation of the molecular ion by ESI-MS. While it is curious that this alkylated pyridinium compound was not observed during the analogous glycosylative transformation en route to trioxacarcin A (see Scheme 3.9), its formation in the present application is nevertheless in keeping with the documented ability of the trioxacarcins to alkylate aromatic *N*-heterocycles such as guanosine residues within duplex DNA (see Scheme 1.2).



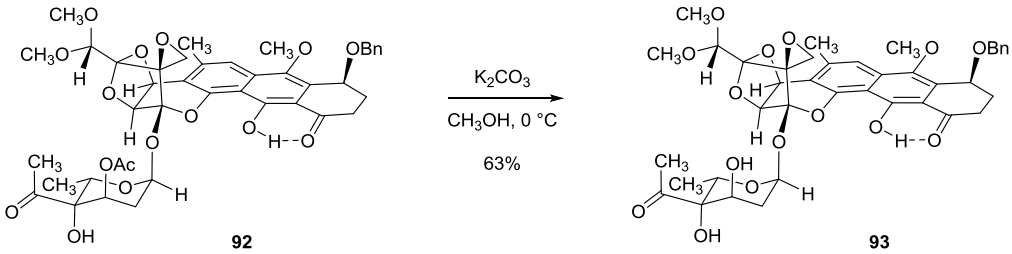

**Scheme 4.9.** Synthesis of 2-Deoxy-DC-45-A1 (**90**), and Observation of Pyridinium Adduct **91**.

The glycosylation methodology developed for the coupling of trioxacarcinose B was used to prepare α-glycoside **92** (50% yield, Scheme 4.10). An efficient two-step deprotection sequence proceeded by hydrogenolysis of the benzyl ether to afford benzylic alcohol **84** (89% yield), followed by methanolysis of the acetate ester within that product (90% yield) to provide triol **85**, a deoxygenated analog of previously synthesized monoglycoside **71** (see Scheme 4.1).




**Scheme 4.10.** Synthesis of 2-Deoxy Analogs Containing Trioxacarcinose B

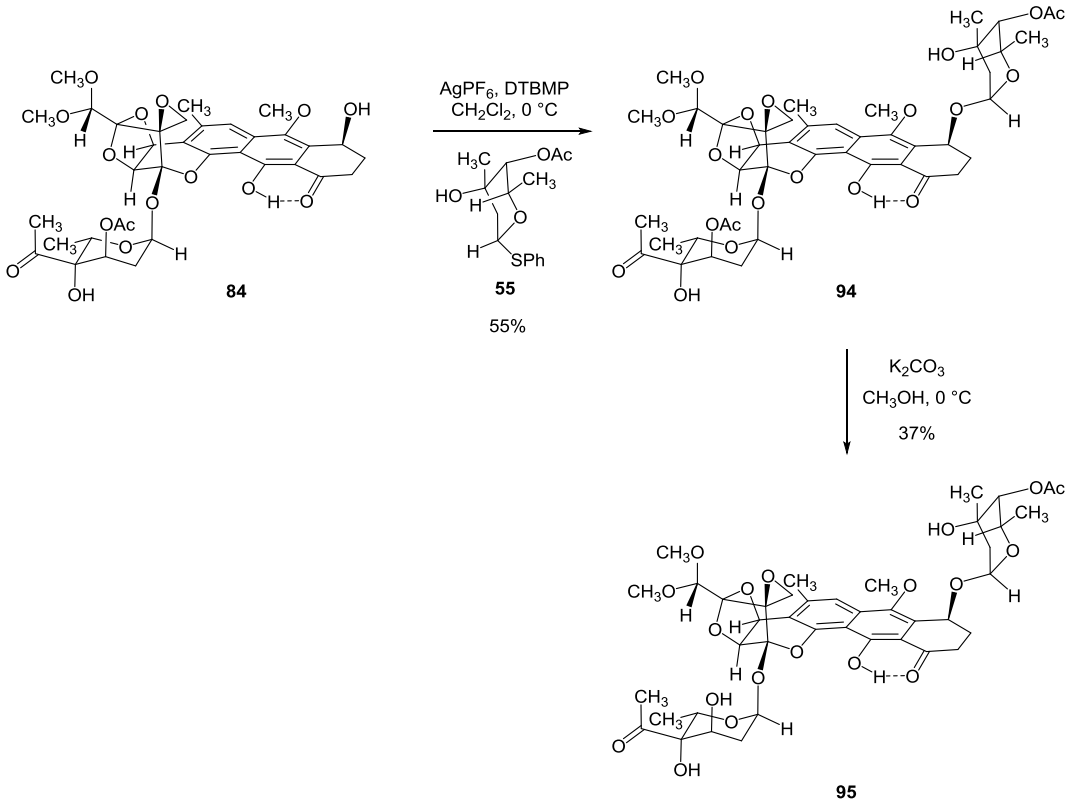
Glycoside product **92** was also deacetylated directly to afford diol benzyl ether analog **93** (Scheme 4.11)




**Scheme 4.11.** Synthesis of Diol Benzyl Ether **93**.

We were particularly interested in the preparation of 2-deoxytrioxacarcin A (**95**) and identified benzylic alcohol **84** as a suitable intermediate for this purpose. To couple a

trioxacarcinose A residue to benzylic alcohol **84**, silver hexafluorophosphate (6.0 equiv) was added to a suspension of benzylic alcohol **84**, 1-phenylthiotrioxacarcinoside A (**55**) (3.0 equiv), 2,6-di-*tert*-butyl-4-methylpyridine (8.0 equiv), and powdered 4-Å molecular sieves in dichloromethane at 0 °C (Scheme 4.12). After 1 h, a solution of triethylamine (50 equiv) in dichloromethane was added, the diluted solution was filtered, and the filtrate was concentrated. Purification of the crude residue by RP-HPLC provided acetyl 2-deoxytrioxacarcin A (**94**) in 55% yield. Deacetylation was accomplished by treatment with potassium carbonate in methanol at 0 °C to afford 2-deoxytrioxacarcin A (**95**) (37% yield).



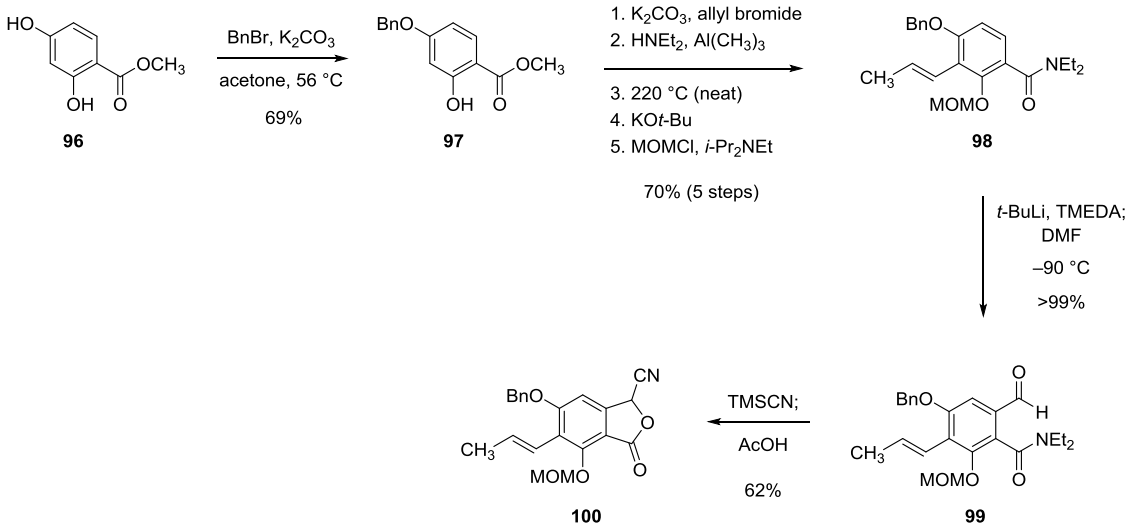

**Scheme 4.12.** Synthesis of 2-Deoxytrioxacarcin A (**95**)

**Modification of the Cyanophthalide Component**

Having created a series of novel trioxacarcin analogs by modification of the enone component **20**, we turned our attention to the modification of other components used in our route to trioxacarcins. I considered analogs which could be prepared by modification of cyanophthalide component **19** and focused on alteration of the methyl substituent present within cyanophthalide **19** (which becomes the C6 methyl group of fully synthetic trioxacarcins). One straightforward modification was introduced in the form of benzyloxy cyanophthalide **100**, which was prepared from methyl 2,4-dihydroxybenzoate (**96**) in an 8-step sequence (Scheme 4.13). Thus, regioselective benzylation of methyl 2,4-dihydroxybenzoate (**96**) was accomplished by treatment with benzyl bromide and potassium carbonate in refluxing acetone to provide benzyl ether **97** in 69% yield.[71] Benzyl ether **97** was transformed to amide **98** by a five-step sequence identical to that used to prepare the analogous amide **29** from 4-methylsalicylic acid in the original synthesis of cyanophthalide component **19** (see Scheme 1.5). In the present application this sequence afforded pure amide **98** in 70% yield over 5 steps, with only a single chromatographic purification at the conclusion of the sequence. Treatment of a solution of amide **98** and tetramethylethylenediamine (TMEDA) in tetrahydrofuran at –90 °C with *tert*-butyllithium accomplished directed *ortho*-lithiation, and trapping of the resulting aryllithium species with *N*,*N*-dimethylformamide provided aldehyde **99** in quantitative yield. Much lower conversion to the aldehyde was observed when the reaction was conducted at –78 °C, and this stands in contrast to the corresponding transformation of methyl-substituted substrate **29** (Scheme 1.5), which proceeds efficiently at –78 °C.

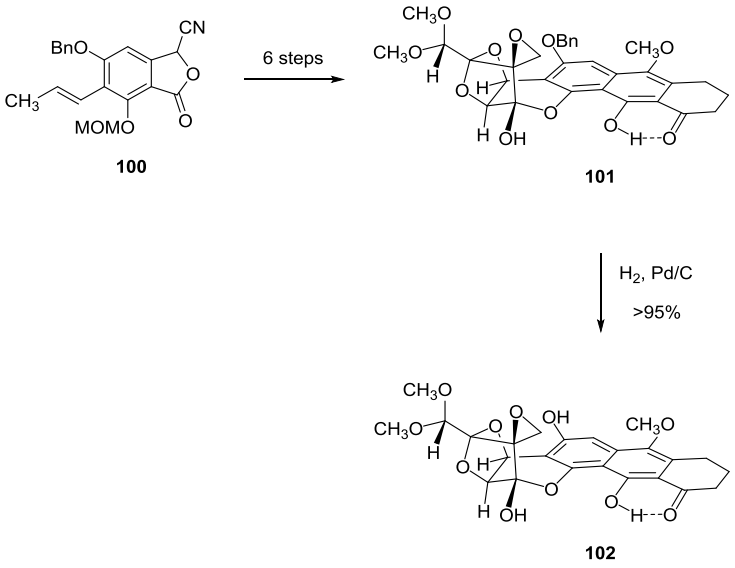
[71] Tangdenpaisal, K.; Sualek, S.; Ruchirawat, S.; Ploypradith, P. *Tetrahedron* **2009**, *65*, 4316–4325.

Aldehyde **99** was treated with cyanotrimethylsilane to produce an intermediate cyanohydrin (not isolated), which upon stirring in glacial acetic acid at 23 °C for 41 h afforded the cyanophthalide **100** (62% yield).



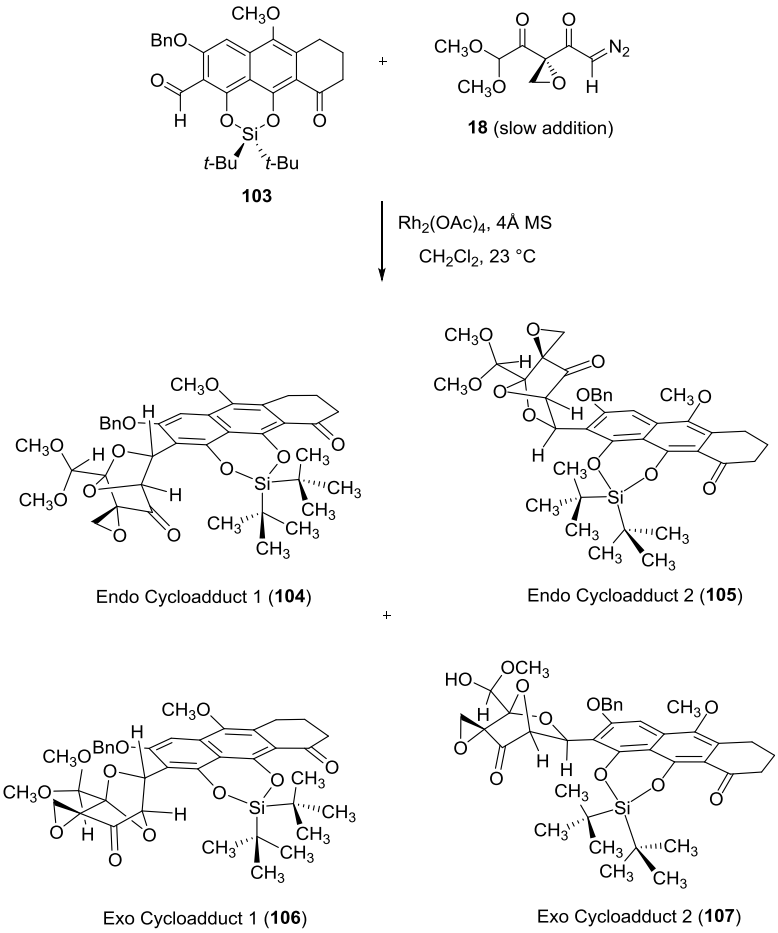

**Scheme 4.13.** Synthesis of Benzyloxy Cyanophthalide **100**.

Benzyloxy cyanophthalide **100**, epoxy diazo diketone **18**, and 2-cyclohexen-1-one were used to assemble benzyl ether **101** (Scheme 4.14) by an analogous sequence to that used for the synthesis of synthetic precursor **23** (see Scheme 1.3). The benzyl ether group was cleaved efficiently by hydrogenolysis to provide unstable phenol analog **102**, which underwent decomposition during attempts at purification by RP-HPLC and upon standing in chloroform, forming a mixture of products (Scheme 4.14).




**Scheme 4.14.** Synthesis of Benzyl Ether Analog **101** and Phenol Analog **102**.

It is interesting to note that the carbonyl ylide–aldehyde cycloaddition used to prepare benzyl ether analog **101** differed in the distribution of diastereomeric cycloadduct products relative to the cycloaddition used to prepare differentially protected DC-45-A2 (see Scheme 1.4), favoring the desired endo cycloadduct 2 (**105**) to a greater extent (Scheme 4.15). The distribution of diastereomers was determined by analysis of the $^{1}$H NMR spectrum of the crude product, assigning the structures of the diastereomers by analogy to those produced in the cycloaddition of aldehyde and epoxy diazo diketone (see Scheme 1.4).[18]




Relative Distribution of Cycloadducts (%)[a]

| Endo 1 (**104**) | Endo 2 (**105**) | Exo 1 (**106**) | Exo 2 (**107**) |
|---|---|---|---|
| 27 | **59** | 7 | 6 |

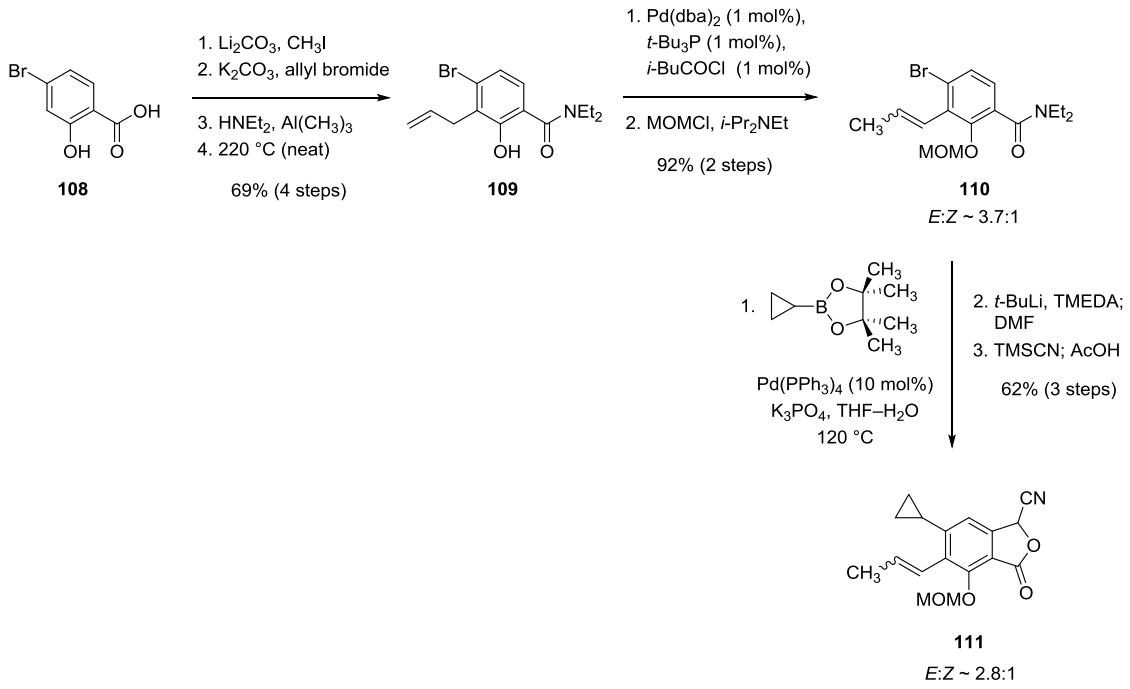
[a] Determined by analysis of the $^1$H NMR spectrum of the crude product mixture

**Scheme 4.15.** Carbonyl Ylide–Aldehyde Cycloaddition between Aldehyde **103** and Diazo Diketone **18**.

I also prepared a second modified cyanophthalide component, cyclopropyl cyanophthalide **111**, by incorporation of a bromide substituent at the outset followed by Suzuki coupling at a later stage (Scheme 4.16). Thus, 4-bromo-2-hydroxybenzoic acid (**108**) was converted to the allyl arene **109** by a four-step sequence identical to that used to prepare the analogous intermediate in the synthesis of cyanophthalide component **19**

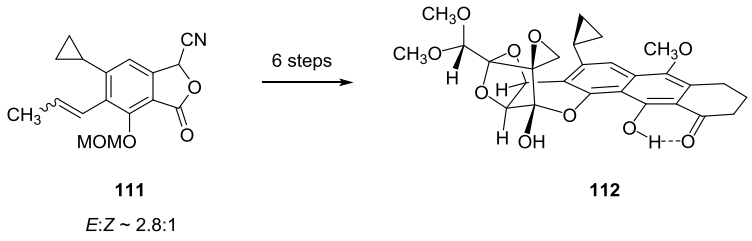
(see Scheme 1.5). This sequence provided **109** in 69% yield over four steps, with a single chromatographic purification step at the conclusion of the sequence. At this stage isomerization of the alkene within intermediate **109** to the internal position was required, but treatment with potassium *tert*-butoxide at elevated temperature—conditions used for the analogous step in the preparation of cyanophthalides **19** and **100**—was not suitable due to the presence of the aryl bromide. Alkene isomerization was accomplished by treatment with bis(dibenzylideneacetone)palladium(0), tri-*tert*-butylphosphine, and isobutyryl chloride, conditions originally reported by Gauthier and coworkers.[72] After protection of the phenol as the corresponding methoxymethyl ether, alkene **110** was obtained in 92% yield over two steps as a ~3.7:1 mixture of *E* and *Z* isomers, respectively (the alkene stereochemistry is inconsequential). The bromide group within intermediate **110** could in principle be converted to a wide variety of novel cyanophthalides. I conducted a Suzuki coupling with cyclopropylboronic acid pinacol ester using tetrakis(triphenylphosphine)palladium(0) as catalyst and potassium phosphate as base in tetrahydrofuran–water at 120 °C. The cyclopropyl arene product of this reaction was subjected to the final two steps originally used to prepare cyanophthalide **19** (without modification) to provide cyclopropyl cyanophthalide **111** in 62% yield over 3 steps. The cyanophthalide product prepared in this manner was a 2.8:1 mixture of *E* and *Z* olefin isomers, respectively.

[72] Gauthier, D.; Lindhardt, A. T.; Olsen, E. P.; Overgaard, J.; Skrydstrup, T. *J. Am. Chem. Soc.* **2010**, *132*, 7998–8009.




**Scheme 4.16.** Synthesis of Cyclopropyl Cyanophthalide **111**.

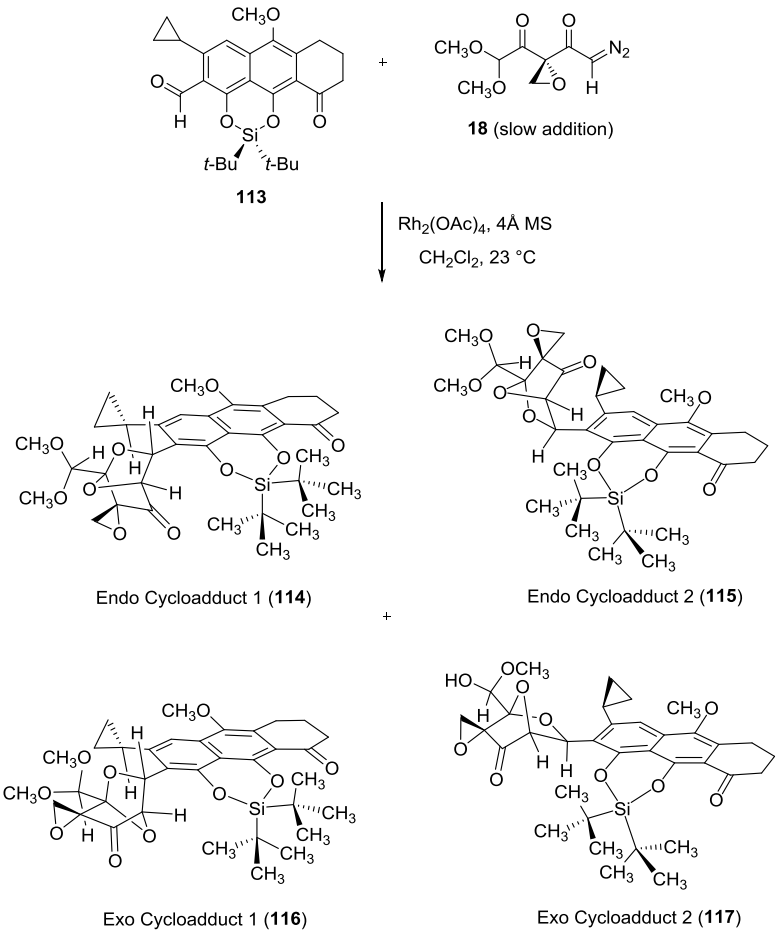
Cyclopropyl cyanophthalide **111**, epoxy diazo diketone **18**, and 2-cyclohexen-1-one were used to assemble cyclopropyl analog **112** (Scheme 4.17) by an analogous sequence to that used for the synthesis of synthetic precursor **23** (see Scheme 1.3).




**Scheme 4.17.** Synthesis of Cyclopropyl Analog **112**.

The carbonyl ylide–aldehyde cycloaddition used to prepare cyclopropyl analog **112** produced the desired endo diastereomer 2 (**115**, 45% of the crude mixture, Scheme 4.18) to only a slightly greater extent than the corresponding cycloaddition used to

prepare differentially protected DC-45-A2 (40% of the crude mixture, see Scheme 1.4). The distribution of diastereomers was determined by analysis of the $^1$H NMR spectrum of the crude product, assigning the structures of the diastereomers by analogy to those produced in the cycloaddition of aldehyde and epoxy diazo diketone (see Scheme 1.4).[18]




Relative Distribution of Cycloadducts (%)[a]

| Endo 1 (**114**) | Endo 2 (**115**) | Exo 1 (**116**) | Exo 2 (**117**) |
|---|---|---|---|
| 35 | **45** | 15 | 6 |

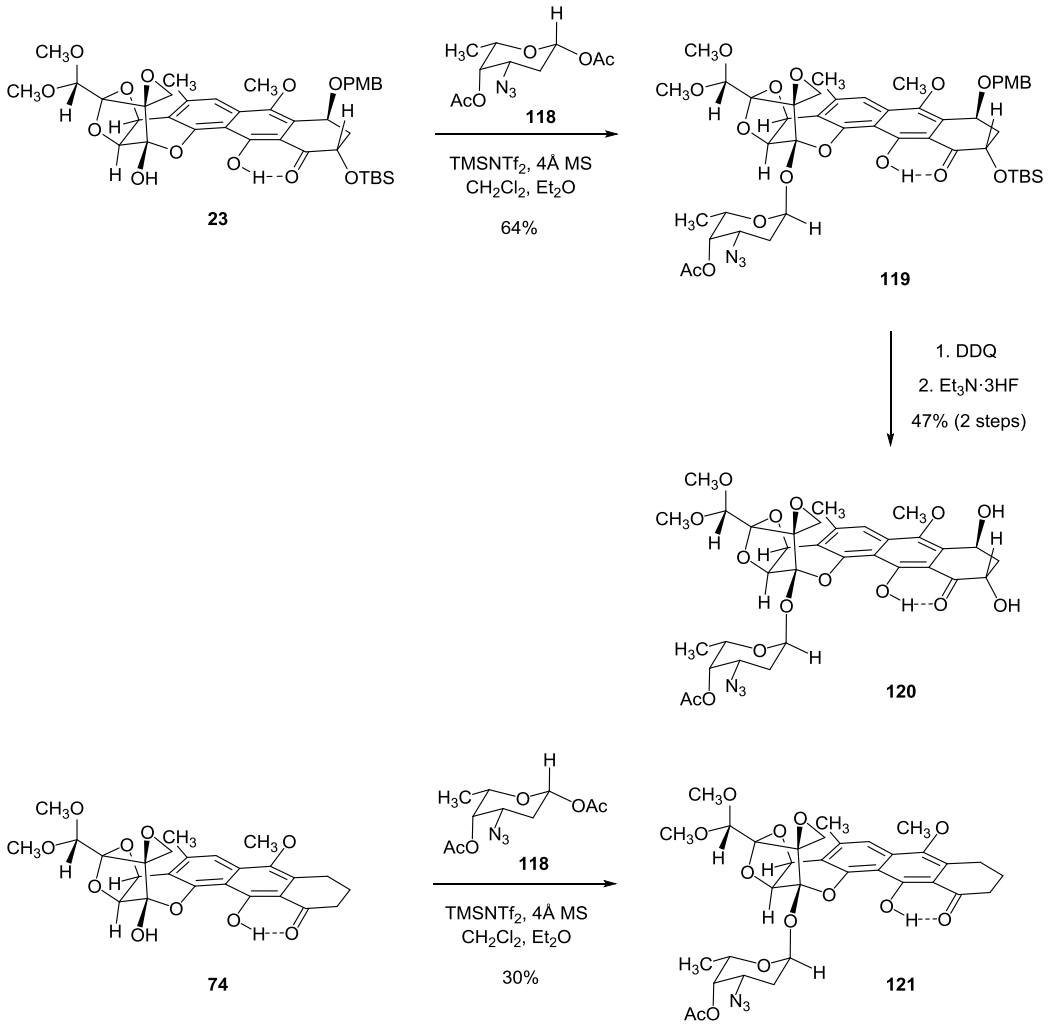
[a] Determined by analysis of the $^1$H NMR spectrum of the crude product mixture

**Scheme 4.18.** Carbonyl Ylide–Aldehyde Cycloaddition between Cyclopropyl Aldehyde **113** and Diazo Diketone **18**.

**Modification of the Glycosyl Components**

We also explored modification of the two glycosidic components used to prepare trioxacarcin A, glycosyl donors derived from trioxacarcinose A (**2**) and trioxacarcinose B (**3**). We successfully incorporated an azido glycoside subunit derived from the deoxysugar daunosamine to prepare a collection of azido glycoside trioxacarcins. In one case, glycosylation of differentially protected synthetic precursor **23** was conducted using 1-*O*-acetyl glycoside **118** as glycosyl donor[73] and *N*-trimethylsilyl-bis(trifluoromethanesulfonyl)imide as the Lewis-acidic promoter. The α-glycoside product **119** was obtained in 64% yield after purification by RP-HPLC (Scheme 4.19). Using the usual two-step deprotection sequence, the PMB ether was cleaved by treatment with DDQ, and the *tert*-butyldimethylsilyl ether within this product was removed by subsequent treatment with triethylamine trihydrofluoride to provide the diol glycoside **120** (47% yield, 2 steps). Glycosylation of dideoxy-DC-45-A2 (**74**) in a similar manner provided glycoside **121**, a 2,4-dideoxy analog of **120**.

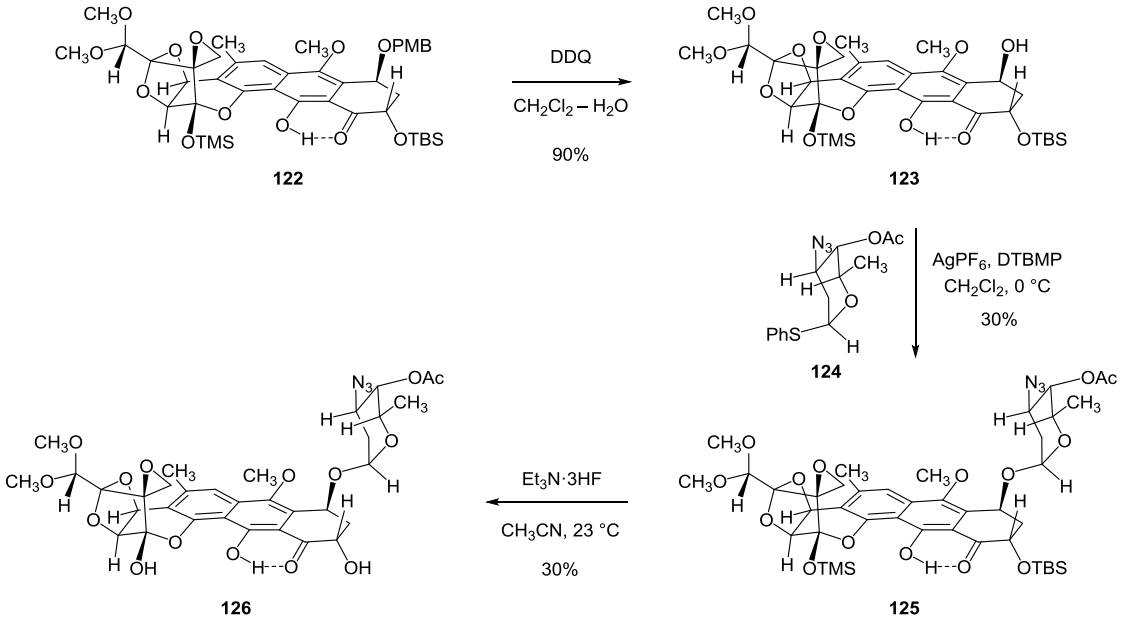
[73] Zhang, G.; Fang, L.; Zhu, L.; Aimiuwu, J. E.; Shen, J.; Cheng, H.; Muller, M. T.; Lee, G. E.; Sun, D.; Wang, P. G. *J. Med. Chem.* **2005**, *48*, 5269–5278.




**Scheme 4.19.** Syntheses of Analogs **120** and **121** Containing an Azido Sugar in Place of Trioxacarcinose B.

We were also successful in coupling the same azido sugar unit to the benzylic alcohol at position C4 of the trioxacarcin scaffold, the position occupied by trioxacarcinose A in the natural products trioxacarcin A (**1**) and DC-45-A1 (**10**). Thioglycoside donor **124**[73] was an appropriate glycosyl donor for this purpose. The glycosylation substrate was prepared from trimethylsilyl hemiketal **122**[62] by cleavage of the PMB ether with DDQ, to afford benzylic alcohol **123** (Scheme 4.20). Coupling of alcohol **123** and thioglycoside **124** was accomplished by treatment with silver

hexafluorophosphate and 2,6-di-*tert*-butyl-4-methylpyridine, conditions which were also employed for the coupling of monoglycoside **67** and 1-phenylthiotrioxacarcinoside A (**55**) in our synthesis of trioxacarcin A (see Scheme 3.9). In the present application we obtained α-glycoside **125** in 30% yield after purification by RP-HPLC. Treatment of glycoside **125** with triethylamine trihydrofluoride effected the desilylation of the hemiketal function as well as cleavage of the *tert*-butyldimethylsilyl ether to afford alcohol **126** (30% yield), an analog of DC-45-A1 (**10**) in which the trioxacarcinose A residue has been replaced with an azido sugar.

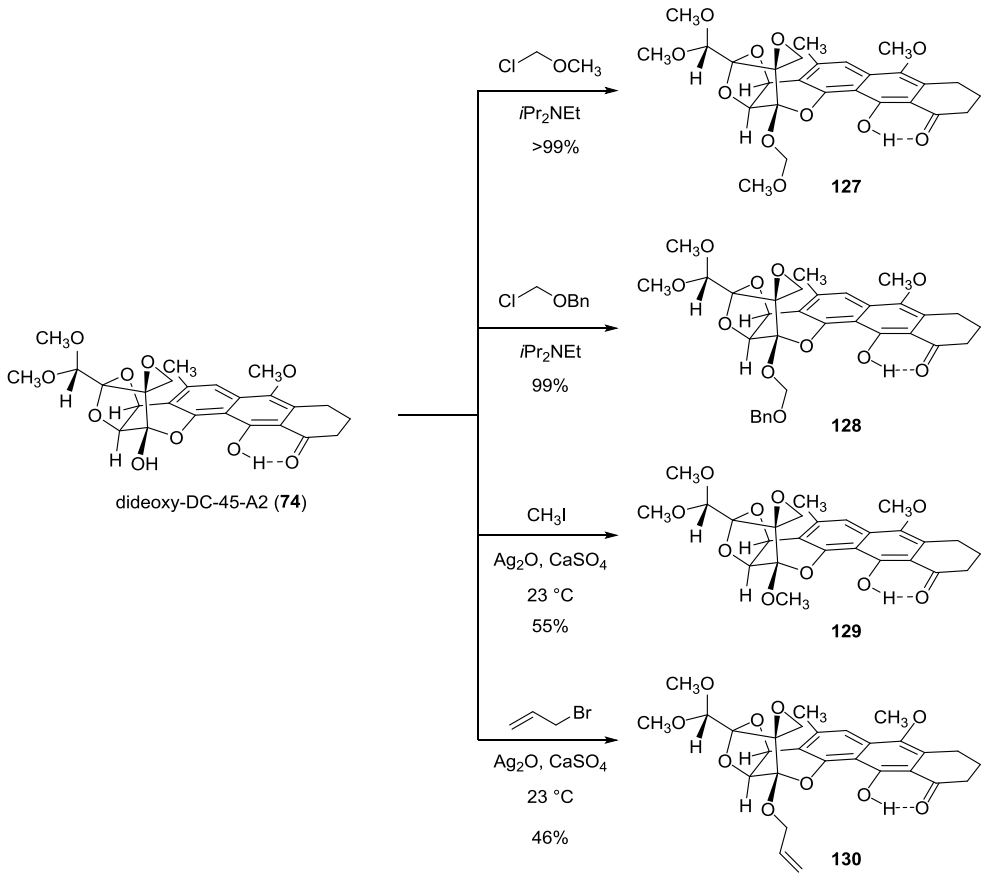



**Scheme 4.20.** Synthesis of Analog **126** Containing an Azido Sugar in Place of Trioxacarcinose A.

**Transformations of Fully Functionalized Trioxacarcins**

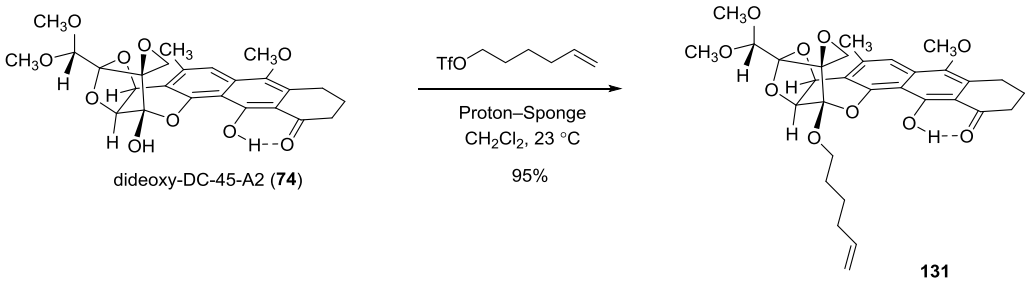
In the previous sections I described the variation of four of the five key building blocks used to prepare trioxacarcin A (the cyanophthalide **19**, the cyclohexenone **20**, the trioxacarcinose B donor **65** and the trioxacarcinose A donor **55**) in order to prepare novel trioxacarcins with deep-seated structural modifications. We also considered whether additional structural variability could be introduced rapidly by late-stage structural modification of fully functionalized trioxacarcins. In this section I describe our identification of various novel forms of reactivity of fully functionalized trioxacarcins which further expands the pool of structures available for study.

We found that the hemiketal function of dideoxy-DC-45-A2 (**74**) undergoes smooth *O*-alkylation reactions with activated carbon nucleophiles. For example, treatment of a solution of dideoxy-DC-45-A2 and *N*,*N*-diisopropylethylamine with chloromethyl methyl ether (MOMCl) in dichloromethane at 23 °C afforded methoxymethylated ketal **127** in quantitative yield after purification by chromatography on silica gel. The alkylation of dideoxy-DC-45-A2 (**74**) with benzyl chloromethyl ether was equally efficient (**128**, 99% yield). Postdoctoral researcher Andreas Schumacher prepared methylated analog **129** by stirring a suspension of dideoxy-DC-45-A2, iodomethane, silver(I) oxide, and calcium sulfate at 23 °C, and use of allyl bromide as the electrophile under similar conditions afforded allylated analog **130**, both in moderate yield. In none of the above alkylation reactions did we observe functionalization of the phenol.




**Scheme 4.21.** *O*-Alkylation Reactions of Dideoxy-DC-45-A2 (**74**)

Furthermore, I found that the hemiketal function of dideoxy-DC-45-A2 (**74**) undergoes clean *O*-alkylation reactions with primary alkyl triflates (Scheme 4.22). For example, dideoxy-DC-45-A2 (**74**) was smoothly *O*-alkylated with 5-hexenyl triflate using 1,8-bis(dimethylamino)naphthalene (Proton–Sponge) as base to afford the terminal alkene-containing product **131** in 95% yield.




**Scheme 4.22.** *O*-Alkylation of Dideoxy-DC-45-A2 (**74**) with a Primary Alkyl Triflate.

## Conclusion

In this chapter I presented the results of efforts related to the synthesis of novel trioxacarcin analogs. Taken together, these represent a thorough examination of the chemistry of the trioxacarcins and an exploration of the capabilities of our component-based synthetic route. Using component modification as the primary (albeit not exclusive) strategy, these studies have resulted in a substantial expansion of the pool of known trioxacarcins, preparing many analogs that would be inaccessible by other means and demonstrating the advantages of a convergent, component-based synthetic strategy for the assembly of a target of interest.

## Experimental Section

*General Experimental Procedures*

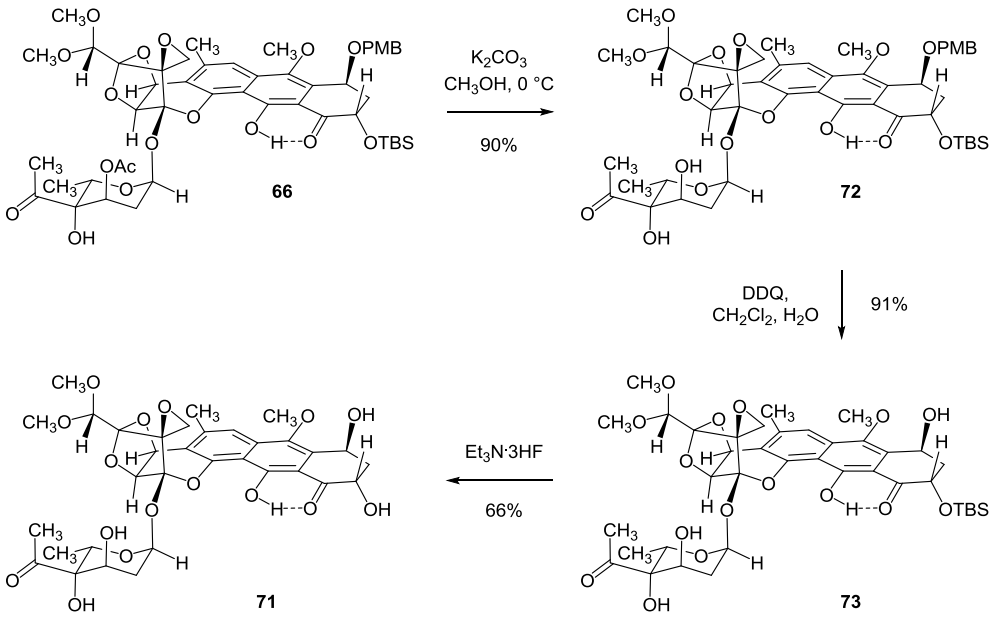
All reactions were performed in round-bottom flasks fitted with rubber septa under a positive pressure of argon, unless otherwise noted. Air- and moisture-sensitive liquids were transferred by syringe or stainless steel cannula. Organic solutions were concentrated by rotary evaporation (house vacuum, ca. 25−40 Torr) at ambient temperature, unless otherwise noted. Analytical thin-layer chromatography (TLC) was performed using glass plates pre-coated with silica gel (0.25 mm, 60 Å pore-size, 230−400 mesh, Merck KGA) impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light, then were stained with either an aqueous sulfuric acid solution of ceric ammonium molybdate (CAM), an acidic solution of *p*-anisaldehyde in ethanol (Anis), or an aqueous sodium hydroxide–potassium carbonate solution of potassium permanganate ($KMnO_4$) followed by brief heating on a hot plate. Flash-column chromatography was performed as described by Still et al.,[52] employing silica gel (60 Å, 32-63 μm, standard grade, Dynamic Adsorbents, Inc.).

*Materials*

Commercial solvents and reagents were used as received with the following exceptions. Tetrahydrofuran, dichloromethane, benzene, toluene, and ether were purified by the method of Pangborn et al.[53] 4-Å molecular sieves (powder, Aldrich) were stored in a laboratory oven at 140 °C and were allowed to cool in a desiccator immediately before use.

*Instrumentation*

Proton magnetic resonance ($^1$H NMR) spectra were recorded on Varian INOVA 500 (500 MHz) or 600 (600 MHz) NMR spectrometers at 23 °C. Proton chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to residual protium in the NMR solvent ($CHCl_3$, δ 7.26). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances, br = broad, app = apparent), integration, and coupling constant (*J*) in Hertz. Carbon nuclear magnetic resonance spectra ($^{13}$C NMR) were recorded on Varian INOVA 500 (125 MHz) NMR spectrometers at 23 °C. Carbon chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to the carbon resonances of the NMR solvent ($CDCl_3$, δ 77.0). Infrared (IR) spectra were obtained using a Shimadzu 8400S FT-IR spectrometer. Data are represented as follows: frequency of absorption ($cm^{-1}$), intensity of absorption (s = strong, m = medium, w = weak, br = broad). Optical rotations were measured on a Jasco DIP-0181 digital polarimeter with a sodium lamp and are reported as follows: $[\alpha]^{T[^\circ C]}_{\lambda}$ (c = g/100 mL, solvent). Circular dichroism spectra were obtained using a Jasco J-710 spectropolarimeter. High-resolution mass spectra were obtained at the Harvard University Mass Spectrometry Facility. High performance liquid chromatography purifications were performed using an Agilent Technologies 1200 Series preparative HPLC system.




Trioxacarcinose B Monoglycoside **71**.

Potassium carbonate (2.6 mg, 0.5 equiv) was added to a solution of α-glycoside **66** (37 mg, 38 μmol, 1 equiv) in methanol (7.7 mL) at 0 °C. After 3 h, the mixture was partitioned between chloroform (100 mL) and saturated aqueous sodium chloride solution (20 mL). The layers were separated. The aqueous layer was extracted with chloroform (2 × 20 mL). The organic layers were combined and the combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the alcohol **72** (32 mg, 90%).

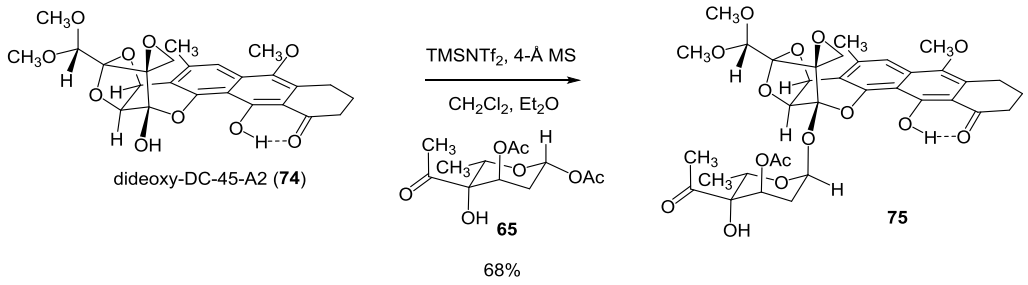
2,3-dichloro-5,6-dicyanobenzoquinone (4.7 mg, 21 μmol, 1.2 equiv) was added to a vigorously stirring, biphasic solution of alcohol **72** (16 mg, 17 μmol, 1 equiv) in dichloromethane (2.2 mL) and water (440 μL) at 23 °C. The reaction flask was covered with aluminum foil to exclude light. Over the course of 2.5 h, the color of the reaction mixture changed from myrtle green to yellow. The product solution was partitioned

between water (10 mL) and dichloromethane (40 mL). The layers were separated. The aqueous layer was extracted with dichloromethane (20 mL). The organic layers were combined and the combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 40→90% acetonitrile in water, flow rate: 15 mL/min) to provide after concentration the pure benzylic alcohol **73** (12.7 mg, 91%) as a yellow-green oil.

Triethylamine–trihydrofluoride (18 μL, 110 μmol, 30 equiv) was added to a solution of benzylic alcohol **73** (3.0 mg, 3.7 μmol, 1 equiv) in acetonitrile (0.5 mL) at 23 °C. The reaction flask was covered with aluminum foil to exclude light. After 21 h, the product solution was partitioned between dichloromethane (50 mL) and saturated aqueous sodium chloride solution (20 mL). The layers were separated. The aqueous layer was extracted with dichloromethane (3 × 10 mL). The organic layers were combined and the combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 20→50% acetonitrile in water, flow rate: 15 mL/min) to provide after concentration the pure trioxacarcinose B monoglycoside **71** (1.7 mg, 66%).

| | |
|---|---|
| **$^1$H NMR**: (500 MHz, $CDCl_3$) | 13.84 (br, 1H), 7.48 (s, 1H), 5.85 (d, 1H, $J$ = 2.6 Hz), 5.46 (t, 1H, $J$ = 2.9 Hz), 5.37 (d, 1H, $J$ = 4.0 Hz), 5.24 (d, 1H, $J$ = 4.0 Hz), 5.02 (q, 1H, $J$ = 6.6 Hz), 4.93 (dd, 1H, $J$ = 12.8, 5.5 Hz), 4.77 (s, 1H), 4.25 (d, 1H, $J$ = 9.5 Hz), |

| | | |
|---|---|---|
| | 4.16 (br, 1H), 3.92 (s, 3H), 3.71 (brd, 1H, $J$ = 8.8 Hz), 3.63 (s, 3H), 3.49 (s, 3H), 3.00 (br, 1H), 2.98 (d, 1H, $J$ = 5.9 Hz), 2.89 (d, 1H, $J$ = 5.9 Hz), 2.74 (ddd, 1H, $J$ = 13.2, 5.5, 2.9 Hz), 2.62 (s, 3H), 2.49 (s, 3H), 2.44 (dt, 1H, $J$ = 14.7, 3.7 Hz), (dt, 1H, $J$ = 13.2, 3.3 Hz), 2.11 (ddd, 1H, $J$ = 14.7, 2.6, 1.5 Hz), 1.09 (d, 3H, $J$ = 6.6 Hz). | |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 210.4, 203.3, 162.4, 151.8, 144.4, 142.6, 135.7, 129.6, 116.6, 114.6, 107.3, 104.7, 101.5, 99.7, 94.9, 79.6, 71.4, 70.2, 69.2, 68.3, 67.7, 63.8, 62.9, 61.9, 56.9, 56.1, 48.2, 45.7, 37.0, 31.6, 27.8, 20.4, 14.5. | |
| **FTIR**, $cm^{-1}$: (neat) | 3431 (br w), 2926 (m), 1713 (m), 1622 (s), 1256 (s), 1103 (s). | |
| **HRMS**: (ESI) | Calcd for $(C_{33}H_{38}O_{16}+Na)^+$ | 713.2052 |
| | Found | 713.2029 |
| **TLC**: (ethyl acetate) | $R_f$ = 0.18 (UV, CAM) | |

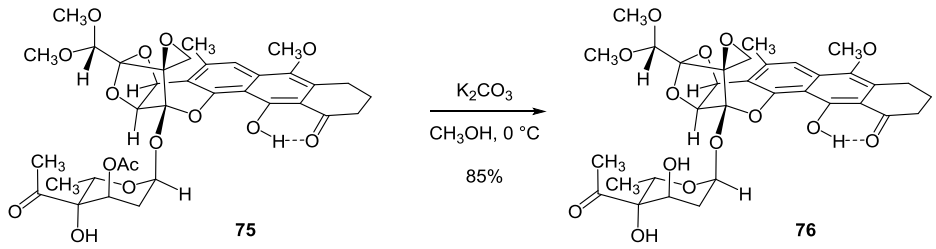



α-Glycoside **75**.

To a solution of dideoxy-DC-45-A2 (**74**)[18] (20.0 mg, 0.041 mmol, 1 equiv) in benzene (2.0 mL) at 23 °C was added 1-*O*-β-acetyl glycoside **5**[65] (56.4 mg, 0.206 mmol, 5.0 equiv). The bright yellow solution was concentrated and the residue was blanketed with argon. Dichloromethane (1.5 mL) and ether (500 μL) were added followed by crushed 4-Å molecular sieves (500 mg). The bright yellow suspension was stirred for 30 min at 23 °C, then was cooled to –78 °C. *N*-(trimethylsilyl)-bis(trifluoromethanesulfonyl)imide (20.2 μL, 0.082 mmol, 2.0 equiv) was added dropwise by syringe over 10 min. After 20 min, a second portion of *N*-(trimethylsilyl)-bis(trifluoromethanesulfonyl)imide (20.2 μL, 0.082 mmol, 2.0 equiv) was added over 10 min. After 2.5 h, the cold mixture was diluted with 15% methanol–dichloromethane (25 mL). Saturated aqueous sodium bicarbonate solution (10 mL) was added. The layers were separated. The aqueous layer was extracted with dichloromethane (25 mL). The organic layers were combined and the combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 40→90% acetonitrile in water, flow rate: 15 mL/min) to provide after concentration the pure α-glycoside **75** (19.7 mg, 68%, in fractions eluting at 22.5–25.5 min) as a bright yellow solid.

| | | |
|---|---|---|
| **$^{1}$H NMR**: (600 MHz, $CDCl_3$) | 14.65 (s, OH). 7.43 (s, 1 H), 5.84 (t, *J* = 3.6 Hz, 1H), 5.29 (d, *J* = 4.2 Hz, 1H), 5.19 (d, *J* = 4.2 Hz, 1H), 5.01 (q, *J* = 6.6 Hz, 1H), 4.75 (t, *J* = 3.6 Hz, 1H), 4.73 (s, 1H), 3.88 (s, OH), 3.77 (s, 3H), 3.63 (s, 3H), 3.47 (s, 3H), 3.03 (app q, *J* = 5.4 Hz, 2H), 2.84 (d, *J* = 6.0 Hz, 1H), 2.77 (d, *J* = 6.0 Hz, 1H), 2.72 (app t, *J* = 6.6 Hz, 2H), 2.58 (s, 3H), 2.36 (s, 3H), 2.33 (app t, *J* = 3.0 Hz, 2H), 2.23 (s, 3H), 2.11-2.06 (m, 2H), 1.08 (d, *J* = 6.0 Hz, 3H). | |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 208.7, 204.3, 170.3, 162.9, 151.8, 142.3, 141.7, 135.2, 130.3, 115.6, 113.2, 113.1, 111.0, 104.5, 101.5, 99.5, 92.3, 78.2, 72.1, 70.7, 69.2, 68.3, 64.4, 60.9, 56.5, 55.8, 47.4, 38.8, 28.9, 27.0, 23.6, 22.1, 21.3, 20.3, 14.5. | |
| **FTIR**, $cm^{-1}$: (neat) | 3482 (w), 2928 (m), 2854 (w), 1740 (s), 1717 (s), 1622 (s), 1449 (m), 1389 (m), 1238 (s), 1096 (s). | |
| **HRMS**: (ESI) | Calcd for $(C_{35}H_{40}O_{15}+Na)^+$ | 723.2259 |
| | Found | 723.2135 |
| **TLC**: (50% ethyl acetate–hexane) | $R_f$ = 0.58 (UV, CAM) | |

**$[\alpha]^{23}_{D}$:** +67.1° (*c* 0.23, $CHCl_3$)




Alcohol **75**.

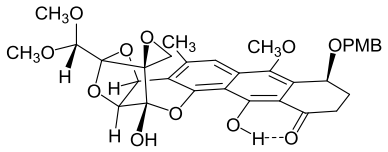
Potassium carbonate (1 mg, 7.24 μmol, 0.34 equiv) was added to a solution of the α-glycoside **75** (15 mg, 0.021 mmol, 1 equiv) in methanol (1 mL) at 0 °C. After 30 min, the cooling bath was removed and the reaction mixture was allowed to warm to 23 °C. After 10 h, the reaction mixture was partitioned between saturated aqueous sodium chloride solution (20 mL) and chloroform (100 mL). The layers were separated. The aqueous layer was extracted with chloroform (3 × 30 mL). The organic layers were combined and the combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 40→90% acetonitrile in water, flow rate: 15 mL/min) to provide after concentration the pure alcohol **76** (12.0 mg, 85%, in fractions eluting at 18.0–24.1 min) as a yellow-orange solid.

**$^1$H NMR**: (600 MHz, $CDCl_3$) 14.69 (s, OH). 7.46 (s, 1 H), 5.86 (d, *J* = 2.4 Hz, 1H), 5.34 (d, *J* = 4.2 Hz, 1H), 5.24 (d, *J* = 3.6 Hz, 1H), 5.02 (q, *J* = 6.6 Hz, 1H), 4.77 (s, 1H), 4.29 (d, *J* = 9.6 Hz, OH), 4.15 (s, OH), 3.77 (s, 3H), 3.71 (dt, *J* = 9.6,3 Hz,

| | |
|---|---|
| | 1H), 3.63 (s, 3H), 3.49 (s, 3H), 3.04 (app q, $J$ = 5.4 Hz, 2H), 2.98 (d, $J$ = 5.4 Hz, 1H), 2.92 (d, $J$ = 5.4 Hz, 1H), 2.73 (app t, $J$ = 7.2 Hz, 2H), 2.59 (s, 3H), 2.50 (s, 3H), 2.43 (t, $J$ = 14.4, 3.6 Hz, 2H), 2.11-2.08 (m, 2H), 1.09 (d, $J$ = 6.6 Hz, 3H). |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 210.5, 204.5, 162.9, 151.6, 142.4, 141.6, 135.2, 130.5, 115.9, 113.1, 113.0, 111.1, 104.6, 101.4, 99.7, 94.8, 79.6, 77.2, 71.5, 70.3, 69.4, 68.4, 63.8, 60.9, 56.8, 56.1, 48.2, 38.8, 27.8, 23.6, 22.1, 20.3, 14.5. FTIR (neat), $cm^{-1}$: 3522 (w), 2930 (s), 2857 (m), 1746 (s), 1717 (s), 1622 (s), 1498 (s), 1456 (s), 1389 (s), 1256 (s), 1095 (s). |
| **FTIR**, $cm^{-1}$: (neat) | 3522 (w), 2930 (s), 2857 (m), 1746 (s), 1717 (s), 1622 (s), 1498 (s), 1456 (s), 1389 (s), 1256 (s), 1095 (s). |
| **HRMS**: (ESI) | Calcd for $(C_{33}H_{38}O_{14}+Na)^+$ 681.2164<br>Found 681.2110 |
| | Calcd for $(C_{33}H_{38}O_{14}+NH_4)^+$ 676.2600<br>Found 676.2530 |
| **TLC**: | $R_f$ = 0.23 (UV, CAM) |

(50% ethyl acetate–hexane)

**$[\alpha]^{23}_{D}$:** +64.0° (*c* 0.60, $CHCl_3$)



78

2-Deoxy Synthetic Precursor **78**.

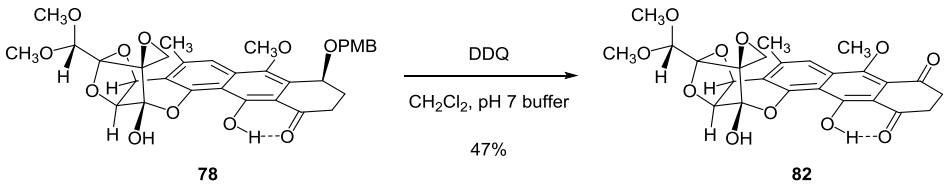
2-Deoxy synthetic precursor **78** was assembled from the epoxy diazo diketone **18**, cyanophthalide **19**, and enone **77** by an analogous sequence to that used to assemble synthetic precursor **23** (see Scheme 1.3).

| | |
|---|---|
| **$^1$H NMR**: (500 MHz, $CDCl_3$) | 15.01 (s, 1H), 7.50 (s, 1H), 7.27 (m, 2H), 6.85 (d, 2H, $J$ = 7.7 Hz), 5.27 (d, 1H, $J$ = 4.0 Hz), 5.15 (s, 1H), 4.83 (d, 1H, $J$ = 4.0 Hz), 4.70 (s, 1H), 4.62 (d, 1H, $J$ = 11.0 Hz), 4.50 (d, 1H, $J$ = 11.4 Hz), 4.37 (s, 1H), 3.83 (s, 3H), 3.79 (s, 3H), 3.62 (s, 3H), 3.47 (s, 3H), 3.16 (m, 1H), 3.13 (d, 1H, $J$ = 5.1 Hz), 3.02 (d, 1H, $J$ = 5.1 Hz), 2.61 (s, 3H), 2.55 (m, 1H), 2.05 (m, 1H). |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 204.2, 162.9, 159.2, 143.7, 141.8, 135.2, 130.3, 129.4, 128.3, 116.5, 114.6, 114.6, 113.8, 109.6, 103.9, 100.1, 98.5, 73.2, 70.3, 69.6, 69.2, 67.5, 62.7, 57.0, 56.6, 55.3, 50.4, 32.3, 26.2, 24.7, 20.4. |
| **FTIR**, $cm^{-1}$: (neat) | 3391 (w), 2928 (m), 1678 (m), 1611 (s), 1389 (s), 1069 (s). |

| | | |
|---|---|---|
| **HRMS**: | Calcd for $(C_{33}H_{34}O_{12}+H)^+$ | 623.2123 |
| (ESI) | Found | 623.2125 |

**TLC**: $R_f$ = 0.55 (UV, CAM)

(60% ethyl acetate–hexane)



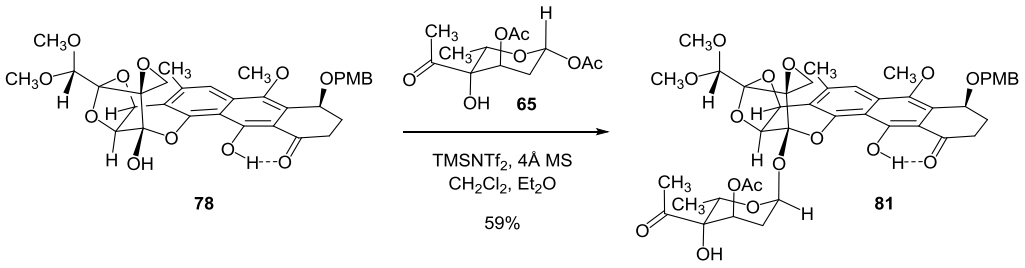

78 82

Diketone **82**.

2,3-dichloro-5,6-dicyanobenzoquinone (4.8 mg, 21 μmol, 1.1 equiv) was added to a solution of 4-methoxybenzyl ether **78** (12 mg, 19 μmol, 1 equiv) in a biphasic mixture of dichloromethane (290 μL) and pH 7 aqueous phosphate buffer solution (29 μL) at 23 °C. The reaction flask was covered with aluminum foil to exclude light. After 1 h, the mixture was partitioned between dichloromethane (40 mL) and water (5 mL). The layers were separated. The organic layer was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 40→90% acetonitrile in water, flow rate: 15 mL/min) to provide after concentration the pure diketone **82** (4.5 mg, 47%).

| | |
|---|---|
| **$^1$H NMR**: (500 MHz, $CDCl_3$) | 15.14 (s, 1H), 7.74 (s, 1H), 5.28 (d, 1H, $J$ = 4.0 Hz), 4.85 (d, 1H, $J$ = 4.0 Hz), 4.71 (s, 1H), 4.43 (br, 1H), 3.95 (s, 3H), 3.62 (s, 3H), 3.48 (s, 3H), 3.17 (d, 1H, $J$ = 5.1 Hz), 3.11–2.98 (m, 5H), 2.64 (s, 3H). |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 202.1, 195.1, 162.7, 151.7, 147.6, 143.2, 141.5, 138.8, 136.4, 121.6, 118.6, 117.4, 104.2, 100.4, 98.9, 73.4, 69.7, |

| | | |
|---|---|---|
| | 69.5, 63.6, 57.3, 56.9, 50.8, 39.6, 36.9, 20.7. | |
| **FTIR**, $cm^{-1}$: (neat) | 3433 (br), 2926 (s), 1701 (m), 1616 (s), 1389 (s), 1107 (s), 1071 (s). | |
| **HRMS**: (ESI) | Calcd for $(C_{25}H_{24}O_{11}+Na)^+$ | 523.1211 |
| | Found | 523.1217 |
| **TLC**: (60% ethyl acetate–hexane) | $R_f$ = 0.24 (UV, CAM) | |

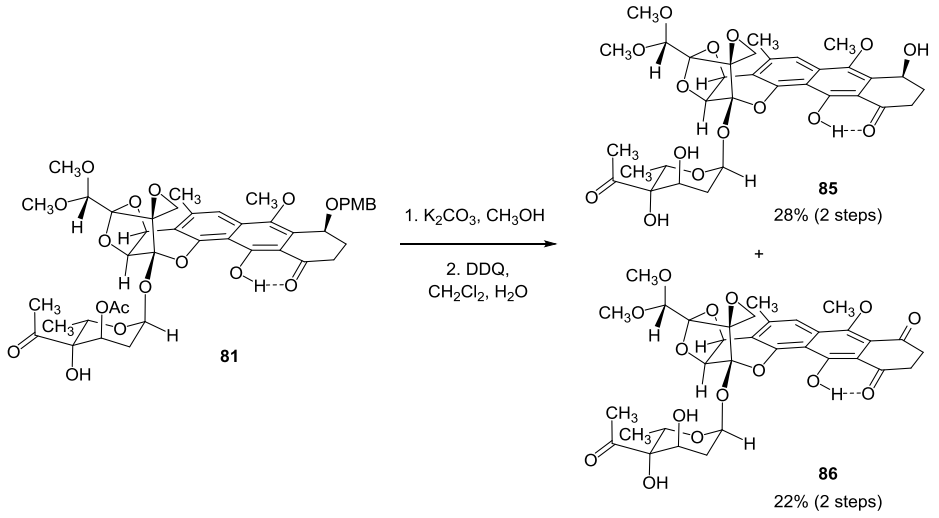



α-Glycoside **81**.

1-*O*-β-Acetyl glycoside **65** (37.0 mg, 0.135 mmol, 6.0 equiv) was added to a solution of the 2-deoxy synthetic precursor **78** (14.0 mg, 0.022 mmol, 1 equiv) in benzene (2.0 mL) at 23 °C. The bright yellow solution was concentrated and the residue was blanketed with argon. Dichloromethane (180 μL) and ether (50 μL) were added, followed by crushed 4-Å molecular sieves (50 mg). The bright yellow suspension was stirred for 60 min at 23 °C, then was cooled to –78 °C. *N*-(trimethylsilyl)bis(trifluoromethanesulfonyl)imide (1.0 M solution in dichloromethane, 90 μL, 0.090 mmol, 4.0 equiv) was added dropwise by syringe over 3 h. After 1 h, triethylamine (12.5 μL, 0.090 mmol, 4.0 equiv) was added at –78 °C. The resulting yellow-orange solution was diluted with 15% methanol–dichloromethane (50 mL) and saturated aqueous sodium bicarbonate solution (10 mL). The cooling bath was removed and the reaction flask was allowed to warm to 23 °C. The layers were separated. The aqueous layer was extracted with dichloromethane (2 × 20 mL). The organic layers were combined. The combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 40→90% acetonitrile in water, flow rate: 15 mL/min) to provide after

concentration the pure α-glycoside **81** (11 mg, 59%, in fractions eluting at 30–33 min) as a yellow-green solid.

| | | |
|---|---|---|
| **$^1$H NMR**: (500 MHz, $CDCl_3$) | 14.72 (s, 1H), 7.48 (s, 1H), 7.27 (d, 2H, $J$ = 7.8 Hz), 6.86 (d, 2H, $J$ = 8.7 Hz), 5.83 (t, 1H, $J$ = 2.8 Hz), 5.29 (d, 1H, $J$ = 4.1 Hz), 5.19 (d, 1H, $J$ = 4.1 Hz), 5.15 (br, 1H), 5.01 (q, 1H, $J$ = 6.4 Hz), 4.75 (t, 1H, $J$ = 3.7 Hz), 4.74 (s, 1H), 4.62 (d, 1H, $J$ = 11.0), 4.51 (d, 1H, $J$ = 11.0), 3.89 (br, 1H), 3.82 (s, 3H), 3.79 (s, 3H), 3.63 (s, 3H), 3.47 (s, 3H), 3.17 (ddd, 1H, $J$ = 18.3, 13.7, 5.0 Hz), 2.83 (d, 1H, $J$ = 6.0 Hz), 2.74 (d, 1H, $J$ = 6.0 Hz), 2.60 (s, 3H), 2.54 (m, 2H), 2.35 (s, 3H), 2.33 (m, 2H), 2.22 (s, 3H), 2.03 (m, 1H), 1.08 (d, 3H, $J$ = 6.4 Hz). | |
| **FTIR**, $cm^{-1}$: (neat) | 2924 (m), 1709 (s), 1614 (m), 1389 (m), 1238 (s). | |
| **HRMS**: (ESI) | Calcd for $(C_{43}H_{48}O_{17}+Na)^+$ | 859.2784 |
| | Found | 859.2771 |
| **TLC**: (60% ethyl acetate–hexane) | $R_f$ = 0.31 (UV, CAM) | |



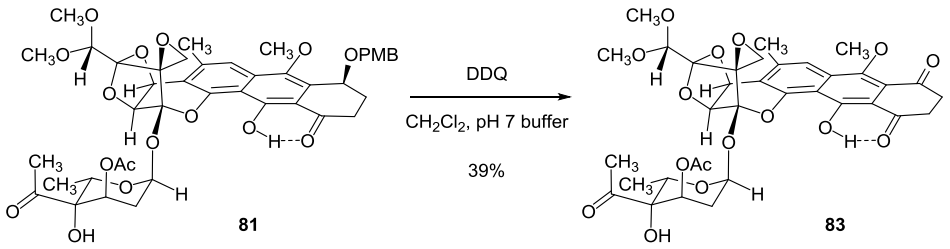

Diketone **86**.

Potassium carbonate (2.5 mg, 18 μmol, 2.0 equiv) was added to an ice-cooled solution of the acetate ester **81** (7.5 mg, 9.0 μmol, 1 equiv) in methanol (0.7 mL). After 140 min, the mixture was partitioned between dichloromethane (20 mL) and saturated aqueous sodium chloride solution (10 mL). The layers were separated. The aqueous layer was extracted with dichloromethane (2 × 20 mL). The organic layers were combined and the combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was dissolved in a biphasic mixture of dichloromethane (333 μL) and water (67 μL) at 23 °C. 2,3-dichloro-5,6-dicyanobenzoquinone (2.3 mg, 10 μmol, 1 equiv) was added. The reaction flask was covered with aluminum foil to exclude light. After 3.5 h, the mixture was partitioned between dichloromethane (50 mL) and water (10 mL). The layers were separated. The aqueous layer was extracted with dichloromethane (3 × 20 mL). The organic layers were combined and the combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm,

gradient elution with 30→80% acetonitrile in water, flow rate: 15 mL/min) to provide, separately, the benzylic alcohol **85** (1.9 mg, 28%) and the diketone **86** (1.5 mg, 22%).

Diketone **86**:

| | | |
|---|---|---|
| **$^1$H NMR**: (500 MHz, $CDCl_3$) | 14.92 (s, 1H), 7.77 (s, 1H), 5.86 (d, 1H, $J$ = 2.8 Hz), 5.39 (d, 1H, $J$ = 4.1 Hz), 5.26 (d, 1H, $J$ = 4.1 Hz), 5.02 (q, 1H, $J$ = 6.4 Hz), 4.77 (s, 1H), 4.16 (br, 1H), 3.95 (s, 3H), 3.72 (s, 1H), 3.63 (s, 3H), 3.50 (s, 3H), 3.10 (m, 2H), 3.00 (m, 3H), 2.91 (d, 1H, $J$ = 5.5 Hz), 2.65 (s, 3H), 2.50 (s, 3H), 2.45 (dt, 1H, $J$ = 14.7, 3.7 Hz), 2.12 (d, 1H, $J$ = 14.7 Hz), 1.10 (d, 3H, $J$ = 6.4 Hz). | |
| **FTIR**, $cm^{-1}$: (neat) | 2924 (s), 1699 (m), 1616 (m), 1389 (m), 1089 (s). | |
| **HRMS**: (ESI) | Calcd for $(C_{33}H_{36}O_{15}+Na)^+$ | 695.1946 |
| | Found | 695.1951 |
| **TLC**: (70% ethyl acetate–hexane) | $R_f$ = 0.28 (UV, CAM) | |

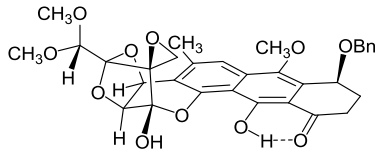



Diketone **83**.

2,3-dichloro-5,6-dicyanobenzoquinone (4.9 mg, 22 μmol, 1.2 equiv) was added to a solution of the 4-methoxybenzyl ether **81** (15 mg, 18 μmol, 1 equiv) in a mixture of dichloromethane (300 μL) and pH 7 aqueous phosphate buffer solution (60 μL) at 23 °C. The reaction flask was covered with aluminum foil to exclude light. After 70 min, the product mixture was partitioned between dichloromethane (40 mL) and water (10 mL). The layers were separated. The aqueous layer was extracted with dichloromethane (20 mL). The organic layers were combined. The combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 20→90% acetonitrile in water, flow rate: 15 mL/min) to provide after the concentration the diketone **83** as an orange powder (5 mg, 39%).

**$^1$H NMR**: (500 MHz, $CDCl_3$) 14.88 (s, 1H), 7.74 (s, 1H), 5.82 (s, 1H), 5.33 (d, 1H, $J$ = 4.0 Hz), 5.21 (d, 1H, $J$ = 4.0 Hz), 5.01 (q, 1H, $J$ = 6.6 Hz), 4.75 (s, 1H), 3.95 (s, 3H), 3.91 (s, 1H), 3.64 (s, 3H), 3.48 (s, 3H), 3.09 (m, 2H), 3.00 (m, 2H), 2.86 (d, 1H, $J$ = 5.9 Hz), 2.76 (d, 1H, $J$ = 5.9 Hz), 2.64 (s, 3H), 2.36 (s,

| | | |
|---|---|---|
| | 3H), 2.35 (m, 2H), 2.23 (s, 3H), 1.09 (d, 3H, $J$ = 6.2 Hz). | |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 208.6, 201.7, 194.8, 170.3, 162.5, 151.7, 147.2, 143.0, 136.2, 121.6, 118.2, 116.8, 110.2, 104.6, 101.8, 99.4, 92.5, 78.1, 72.0, 70.7, 68.9, 68.3, 64.5, 63.4, 56.6, 55.9, 47.4, 39.4, 36.7, 29.7, 28.9, 27.0, 21.3, 20.3, 14.5. | |
| **FTIR**, $cm^{-1}$: (neat) | 2930 (m), 1715 (m), 1645 (m), 1510 (m), 1364 (m), 1105 (s), 835 (s). | |
| **HRMS**: (ESI) | Calcd for $(C_{35}H_{38}O_{16}+NH_4)^+$ | 734.2558 |
| | Found | 734.2593 |
| **TLC**: (70% ethyl acetate–hexane) | $R_f$ = 0.34 (UV, CAM) | |



**88**

2-Deoxy Synthetic Precursor **88**.[74]

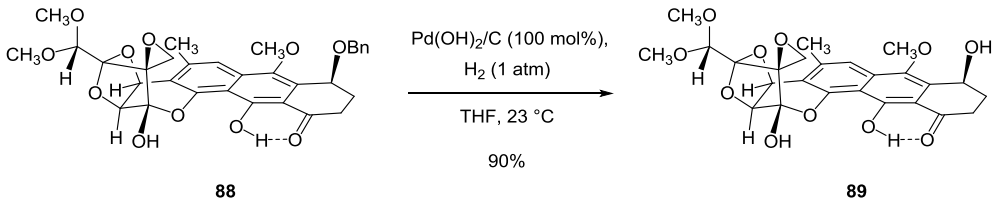
2-Deoxy synthetic precursor **88** was assembled from the epoxy diazo diketone **18**, cyanophthalide **19**, and (*S*)-4-(benzyloxy)cyclohex-2-enone (**87**) by an analogous sequence to that used for the synthesis of synthetic precursor **23** (see Scheme 1.3).

**$^1$H NMR**: (500 MHz, $CDCl_3$) 14.98 (s, 1H), 7.43 (s, 1H), 7.35–7.27 (m, 5H), 5.24 (d, 1H, $J$ = 4.0 Hz), 5.17 (br t, 1H, $J$ = 2.8 Hz), 4.88 (d, 1H, $J$ = 4.4 Hz), 4.70 (s, 1H), 4.69 (d, 1H, $J$ = 12.3 Hz), 4.56 (d, 1H, $J$ = 11.9 Hz), 4.54 (br s, 1H), 3.82 (s, 3H), 3.63 (s, 3H), 3.48 (s, 3H), 3.18 (ddd, 1H, $J$ = 18.3, 13.6, 5.4 Hz), 3.10 (d, 1H, $J$ = 5.2 Hz), 3.00 (d, 1H, $J$ = 5.2 Hz), 2.62– 2.53 (m, 2H), 2.57 (s, 3H), 2.09 (tdd, 1H, $J$ = 14.0, 4.6, 2.0 Hz).

**$^{13}$C NMR**: (125 MHz, $CDCl_3$) 204.2, 162.9, 151.4, 143.8, 141.8, 138.2, 135.1, 128.3, 128.1, 127.7, 127.6, 116.4, 114.6, 114.6, 109.5, 103.8, 100.1, 98.5, 73.2, 70.6, 69.6, 69.2, 67.7, 62.8, 57.0, 56.6, 50.4, 32.3, 26.3, 20.4.

[74] Dr. Andreas Schumacher provided assistance in the large-scale synthesis of 2-deoxy synthetic precursor **88**, which I acknowledge with appreciation.

| | | |
|---|---|---|
| **FTIR**, $cm^{-1}$: | 2918 (s), 1740 (s), 1620 (s), 1389 (s), 1236 (s), 1084 (s). | |
| (neat) | | |
| **HRMS**: | Calcd for $(C_{32}H_{32}O_{11}+Na)^+$ | 615.1837 |
| (ESI) | Found | 615.1842 |
| **TLC**: | $R_f$ = 0.34 (UV, CAM) | |
| (67% ethyl acetate–hexane) | | |




88 89

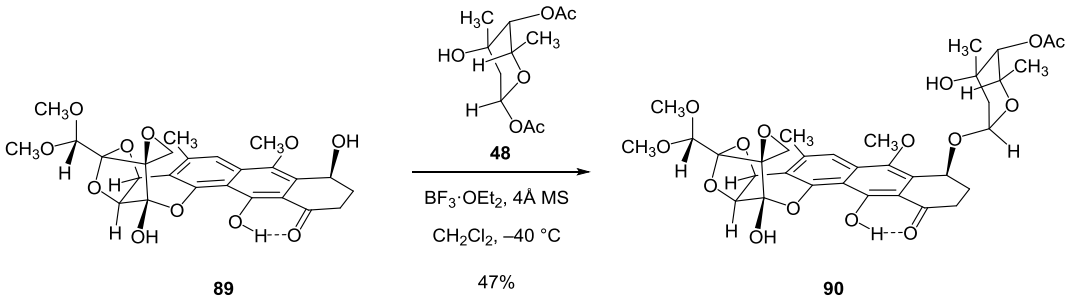
2-Deoxy-DC-45-A2 (**89**).

Palladium hydroxide on carbon (20 wt. %, 18 mg, 0.025 mmol, 1.0 equiv) was added to a solution of 2-deoxy synthetic precursor **88** (15 mg, 0.025 mmol, 1 equiv) in tetrahydrofuran (1.2 mL) at 23 °C. An atmosphere of hydrogen was maintained by sparging with a slow stream of pure hydrogen gas through a 22-gauge stainless steel needle. After 2 h, the mixture was diluted with ethyl acetate (20 mL) and filtered through a short pad of Celite. The filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 40→90% acetonitrile in water, flow rate: 15 mL/min) to provide after concentration pure 2-deoxy-DC-45-A2 (**89**) (11.4 mg, 90%).

**$^1$H NMR**: (500 MHz, $CDCl_3$) 14.94 (s, 1H), 7.42 (s, 1H), 5.39 (t, 1H, $J$ = 3.3 Hz), 5.24 (d, 1H, $J$ = 4 Hz), 4.86 (d, 1H, $J$ = 4 Hz), 4.74 (br s, 1H), 4.70 (s, 1H), 3.91 (s, 3H), 3.61 (s, 3H), 3.46 (s, 3H), 3.10 (ddd, 1H, $J$ = 17.6, 12.1, 5.5 Hz), 3.09 (d, 1H, $J$ = 5.1 Hz), 3.04 (d, 1H, $J$ = 5.1 Hz), 2.75 (br s, 1H), 2.62 (t, 1H, $J$ = 4.2 Hz), 2.58 (s, 3H), 2.26 (m, 2H).

**$^{13}$C NMR**: 203.9, 162.8, 151.5, 143.6, 142.1, 135.3, 130.2, 116.2,

| | | |
|---|---|---|
| (125 MHz, $CDCl_3$) | 114.7, 114.5, 109.0, 103.9, 100.1, 98.6, 73.2, 69.6, 69.2, 62.7, 62.2, 57.0, 56.6, 50.3, 32.7, 29.1, 20.4. | |
| **FTIR**, $cm^{-1}$: (neat) | 3437 (br w), 2926 (m), 1717 (m), 1620 (s), 1389 (s), 1065 (s), 978 (s). | |
| **HRMS**: (ESI) | Calcd for $(C_{25}H_{26}O_{11}+H)^+$ | 503.1548 |
| | Found | 503.1555 |
| **TLC**: (5% methanol–dichloromethane) | $R_f$ = 0.47 (UV, CAM) | |

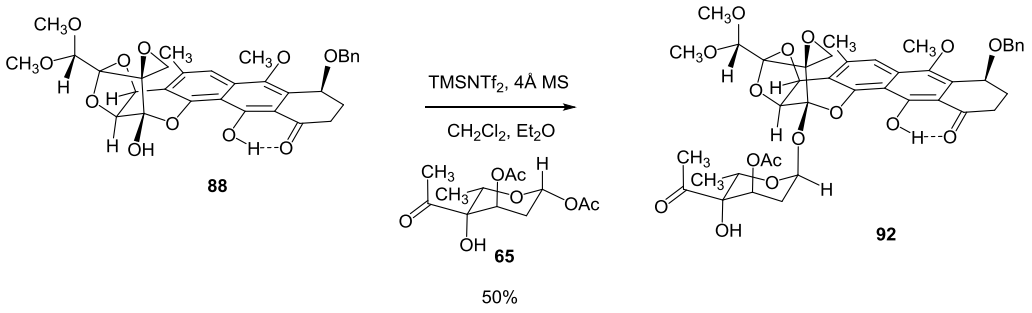



2-Deoxy-DC-45-A1 (**90**).

Boron trifluoride etherate (1.4 μL, 11 μmol, 1.0 equiv) was added to a suspension of 2-deoxy-DC-45-A2 (**89**) (5.7 mg, 11 μmol, 1 equiv), 1-*O*-acetyltrioxacarcinose A (**48**) (5.6 mg, 23 μmol, 2.0 equiv, a ~1:12 mixture of α- and β-anomers, respectively), and powdered 4-Å molecular sieves (~30 mg) in dichloromethane (380 μL) at –40 °C. After 5 min, saturated aqueous sodium bicarbonate solution (1 mL) was added rapidly. The cooing bath was removed and the reaction flask was allowed to warm to 23 °C. The mixture was partitioned between dichloromethane (40 mL) and saturated aqueous sodium bicarbonate solution (9 mL). The layers were separated. The organic layer was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 40→90% acetonitrile in water, flow rate: 15 mL/min) to provide pure 2-deoxy-DC-45-A1 (**90**) as a yellow-green powder (3.7 mg, 47%).

| | |
|---|---|
| **$^1$H NMR**: (500 MHz, $CDCl_3$) | 15.03 (s, 1H), 7.50 (s, 1H), 5.37 (app s, 1H), 5.26 (d, 2H, *J* = 4 Hz), 4.83 (d, 1H, *J* = 4 Hz), 4.73 (s, 1H), 4.71 (s, 1H), 4.49 (q, 1H, *J* = 6.5 Hz), 4.38 (s, 1H), 4.08 (s, 1H), |

| | | |
|---|---|---|
| | 3.85 (s, 3H), 3.62 (s, 3H), 3.47 (s, 3H), 3.16 (d, 1H, $J$ = 5.5 Hz), 3.08 (s, 1H), 3.01 (ddd, 1H, $J$ = 18.7, 13.9, 5.1 Hz), 2.68 (dd, 1H, $J$ = 18.3, 3.7 Hz), 2.61 (s, 3H), 2.46–2.40 (m, 1H), 2.27–2.19 (m, 1H), 2.13 (s, 3H), 1.93 (dd, 1H, $J$ = 14.6, 3.7 Hz), 1.59 (d, 1H, $J$ = 14.6 Hz), 1.22 (d, 3H, $J$ = 6.2 Hz), 1.06 (s, 3H). | |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 203.2, 170.4, 163.3, 151.7, 144.1, 142.3, 135.1, 126.7, 116.8, 114.9, 114.9, 109.2, 103.9, 100.1, 98.5, 97.0, 74.5, 73.2, 69.6, 69.3, 68.8, 66.3, 62.8, 62.7, 57.1, 56.6, 50.6, 36.5, 32.4, 28.3, 25.6, 20.9, 20.4, 16.9. | |
| **FTIR**, $cm^{-1}$: (neat) | 3514 (br w), 2926 (m), 1746 (m), 1620 (m), 1391 (s), 1236 (s), 1084 (s), 997 (s). | |
| **HRMS**: (ESI) | Calcd for $(C_{34}H_{40}O_{15}+Na)^+$ | 711.2259 |
| | Found | 711.2242 |
| **TLC**: (5% methanol–dichloromethane) | $R_f$ = 0.43 (UV, CAM) | |




α-Glycoside **92**.[75]

1-*O*-β-Acetyl glycoside **65**[65] (301 mg, 1.10 mmol, 5.0 equiv) was added to a solution of 2-deoxy synthetic precursor **88** (130 mg, 0.219 mmol, 1 equiv) in benzene (2.0 mL) at 23 °C. The bright yellow solution was concentrated and the residue was blanketed with argon. Dichloromethane (3 mL) and ether (600 μL) were added, followed by crushed 4-Å molecular sieves (1.2 g). The bright yellow suspension was stirred for 90 min at 23 °C, then was cooled to –78 °C. Trimethylsilyl trifluoromethanesulfonate (120 μL, 0.659 mmol, 3.0 equiv) was added dropwise by syringe over 10 min. After 2 h, a second portion of trimethylsilyl trifluoromethanesulfonate (40 μL, 0.220 mmol, 1.0 equiv) was added dropwise by syringe over 10 min. After 3.5 h, triethylamine (122 μL, 0.878 mmol, 4.0 equiv) was added. The suspension was diluted with dichloromethane (50 mL) and filtered through a pad of Celite. Saturated aqueous sodium bicarbonate solution (10 mL) was added, and the layers were separated. The aqueous layer was extracted with dichloromethane (3 × 20 mL). The organic layers were combined and the combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 40→90%

[75] This procedure was conducted by Dr. Andreas Schumacher, whom I acknowledge with appreciation.

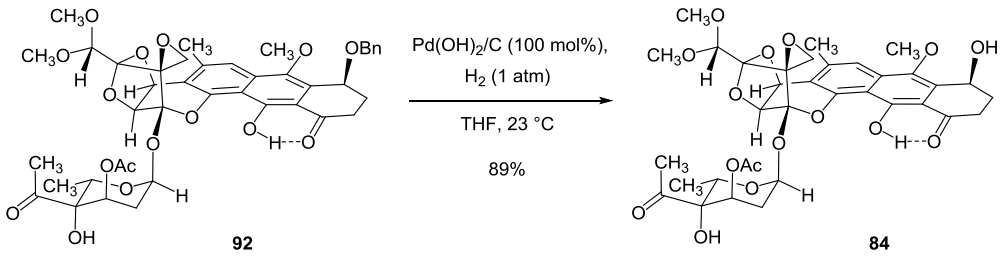
acetonitrile in water, flow rate: 15 mL/min) to provide after concentration the pure α-glycoside **92** (88 mg, 50%, in fractions eluting at 22.5–25.5 min) as a bright yellow solid.

| | |
|---|---|
| **$^1$H NMR**: (500 MHz, $CDCl_3$) | 14.74 (s, 1H), 7.49 (s, 1H), 7.35–7.26 (m, 5H), 5.84 (t, 1H, *J* = 3 Hz), 5.31–5.29 (m, 2H), 5.2 (d, 1H, *J* = 4.0 Hz), 5.17 (app s, 1H), 5.02 (q, 1H, *J* = 6.4 Hz), 4.76 (t, 1H, *J* = 2.8 Hz), 4.75 (s, 1H), 4.7 (d, 1H, *J* = 11.5 Hz), 4.58 (d, 1H, *J* = 11.5 Hz), 3.94 (s, 1H), 3.84 (s, 3H), 3.65 (s, 3H), 3.47 (s, 3H), 3.19 (ddd, 1H, *J* = 18.1, 13.5, 5.0 Hz), 2.83 (d, 1H, *J* = 6.0 Hz), 2.75 (d, 1H, *J* = 6.0 Hz), 2.61 (s, 3H), 2.56 (m, 1H), 2.36 (s, 3H), 2.36–2.33 (m, 2H), 2.23 (s, 3H), 2.06 (m, 1H), 1.09 (d, 3H, *J* = 6.4 Hz). |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 208.6, 204.0, 170.1, 162.9, 151.7, 143.7, 141.8, 138.1, 135.0, 128.2, 128.1, 127.7, 127.5, 116.2, 114.6, 114.2, 109.4, 104.4, 101.4, 99.3, 92.2, 78.0, 71.9, 70.5, 69.0, 68.1, 67.6, 64.3, 62.7, 56.4, 55.8, 47.3, 32.3, 28.7, 26.9, 26.4, 21.2, 20.2, 14.4. |
| **FTIR**, $cm^{-1}$: (neat) | 3404 (w), 2932 (m), 1717 (m), 1620 (s), 1389 (s), 1067 (s). |
| **HRMS**: | Calcd for $(C_{42}H_{46}O_{16} + Na)^+$ 829.2678 |

| | | |
|---|---|---|
| (ESI) | Found | 829.2680 |

**TLC**: $R_f$ = 0.58 (UV, CAM)

(50% ethyl acetate–hexane)




Benzylic alcohol **84**.[75]

Palladium hydroxide on carbon (20 wt. %, 18 mg, 0.025 mmol, 1.0 equiv) was added to a solution of α-glycoside **92** (20 mg, 0.025 mmol, 1 equiv) in tetrahydrofuran (1.2 mL) at 23 °C. An atmosphere of hydrogen was maintained by sparging with a slow stream of pure hydrogen gas through a 22-gauge stainless steel needle. After 30 min, the mixture was diluted with ethyl acetate (20 mL) and filtered through a short pad of Celite. The filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 40→100% acetonitrile in water, flow rate: 15 mL/min) to provide after concentration the pure benzylic alcohol **84** (16 mg, 89%).

**$^1$H NMR**: (500 MHz, $CDCl_3$) 14.71 (s, 1H), 7.45 (s, 1H), 5.81 (t, 1H, *J* = 2.9 Hz), 5.39 (br s, 1H), 5.29 (d, 1H, *J* = 4.1 Hz), 5.19 (d, 1H, *J* = 4.1 Hz), 5.00 (q, 1H, *J* = 6.3 Hz), 4.74 (m, 2H), 3.90 (s, 3H), 3.63 (s, 3H), 3.47 (s, 3H), 3.10 (ddd, 1H, *J* = 17.3, 11.9, 5.2 Hz), 2.83 (d, 1H, *J* = 5.8 Hz), 2.75 (d, 1H, *J* = 5.8 Hz), 2.68 (d, 1H, *J* = 2.1 Hz), 2.63–2.60 (m, 1H), 2.60 (s, 3H), 2.35 (s, 3H), 2.33–2.27 (m, 3H), 2.24–2.22 (m, 1H),

2.22 (s, 3H), 1.07 (d, 3H, $J$ = 6.5 Hz).

| | |
|---|---|
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 208.7, 203.7, 170.2, 163.0, 151.8, 143.4, 142.1, 135.2, 130.3, 116.0, 114.5, 114.3, 109.0, 104.5, 101.6, 99.4, 92.3, 78.2, 72.1, 70.7, 69.1, 68.3, 64.5, 62.7, 62.3, 56.5, 55.8, 47.4, 32.8, 29.2, 28.9, 27.0, 21.3, 20.3, 14.5. |
| **FTIR**, $cm^{-1}$: (neat) | 3462 (w), 2926 (m), 1738 (m), 1618 (s), 1389 (s), 1236 (s), 1099 (s), 982 (s). |

| | | |
|---|---|---|
| **HRMS**: (ESI) | Calcd for $(C_{35}H_{40}O_{16}+H)^+$ | 717.2389 |
| | Found | 717.2403 |

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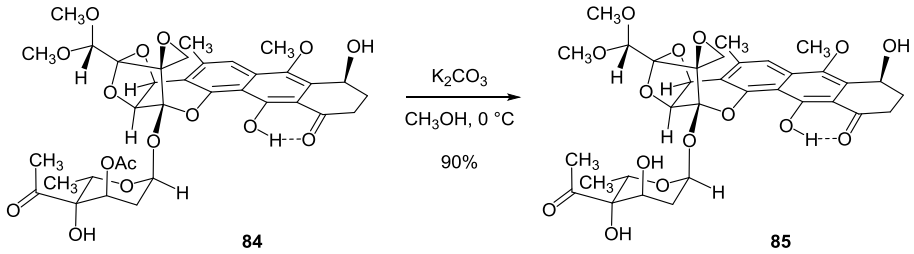
|---|---|
| **TLC**: (67% ethyl acetate–hexane) | $R_f$ = 0.18 (UV, CAM) |

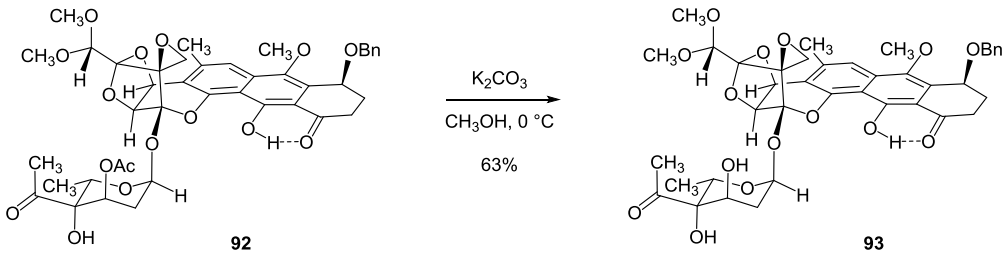



Triol **85**.[75]

Potassium carbonate (0.5 mg, 4 μmol, 0.3 equiv) was added to an ice-cooled solution of benzylic alcohol **84** (8.0 mg, 11 μmol, 1 equiv) in methanol (1.0 mL). After 30 min, the cooling bath was removed and the reaction flask was allowed to warm to 23 °C. After 2.5 h, the mixture was partitioned between chloroform (40 mL) and saturated aqueous sodium chloride solution (10 mL). The layers were separated. The aqueous layer was extracted with chloroform (3 × 20 mL). The organic layers were combined and the combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 40→90% acetonitrile in water, flow rate: 15 mL/min) to provide after concentration the pure triol **85** (6.8 mg, 90%, in fractions eluting at 6–11 min) as a yellow solid.

**$^1$H NMR**: (500 MHz, $CDCl_3$) 14.75 (s, 1H), 7.48 (s, 1H), 5.85 (d, 1H, $J$ = 2.5 Hz), 5.41 (t, 1H, $J$ = 3.9 Hz), 5.35 (d, 1H, $J$ = 4.1 Hz), 5.24 (d, 1H, $J$ = 4.1 Hz), 5.02 (q, 1H, $J$ = 6.4 Hz), 4.77 (s, 1H), 4.16 (s, 1H), 3.92 (s, 3H), 3.71 (br s, 1H), 3.63 (s, 3H), 3.49 (s, 3H), 3.13 (ddd, 1H, $J$ = 17.3, 12.1, 5.2 Hz), 2.98 (d, 1H, $J$ = 5.7 Hz), 2.91 (d, 1H, $J$ = 5.7 Hz), 2.65–2.60 (m,

| | | |
|---|---|---|
| | 1H), 2.61 (s, 3H), 2.50 (s, 3H), 2.43 (dt, 1H, $J$ = 14.5, 3.5 Hz), 2.32 (dq, 1H, $J$ = 14.0, 4.7 Hz), 2.23 (m, 1H), 2.12 (ddd, 1H, $J$ = 14.6, 2.5, 1.4 Hz), 1.09 (d, 3H, $J$ = 6.4 Hz). | |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 210.4, 203.9, 162.9, 151.7, 143.5, 142.0, 135.3, 130.5, 116.3, 114.2, 110.7, 109.1, 104.6, 101.5, 99.7, 94.8, 79.6, 71.5, 70.2, 69.3, 68.3, 63.8, 62.7, 62.3, 56.8, 56.1, 48.2, 32.8, 31.5, 29.2, 27.8, 20.3, 14.5. | |
| **FTIR**, $cm^{-1}$: (neat) | 3514 (m), 2928 (m), 1620 (s), 1389 (s), 1105 (s), 984 (s). | |
| **HRMS**: (ESI) | Calcd for $(C_{38}H_{38}O_{15}+H)^+$ | 675.2283 |
| | Found | 675.2279 |
| **TLC**: (75% ethyl acetate–hexane) | $R_f$ = 0.22 (UV, CAM) | |




92 93

Diol **93**.[75]

Potassium carbonate (0.3 mg, 2 μmol, 0.3 equiv) was added to an ice-cooled solution of α-glycoside **92** (5 mg, 6 μmol, 1 equiv) in methanol (1.0 mL). After 30 min, the cooling bath was removed and the reaction flask was allowed to warm to 23 °C. After 2 h, the mixture was partitioned between chloroform (40 mL) and saturated aqueous sodium chloride solution (10 mL). The layers were separated. The aqueous layer was extracted with chloroform (3 × 20 mL). The organic layers were combined and the combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 40→90% acetonitrile in water, flow rate: 15 mL/min) to provide after concentration the pure diol **93** (3 mg, 63%, in fractions eluting at 28–30 min) as a yellow solid.

**$^1$H NMR**: (500 MHz, $CDCl_3$) 14.76 (s, 1H), 7.52 (s, 1H), 7.36–7.28 (m, 5H), 5.86 (br d, 1H, $J$ = 2.3 Hz), 5.34 (d, 1H, $J$ = 4.4 Hz), 5.24 (d, 1H, $J$ = 4.4 Hz), 5.17 (br s, 1H), 5.02 (q, 1H, $J$ = 6.3 Hz), 4.76 (s, 1H), 4.7 (d, 1H, $J$ = 11.5 Hz), 4.58 (d, 1H, $J$ = 11.5 Hz), 4.26 (d, 1H, $J$ = 9.5 Hz), 4.15 (s, 1H), 3.82 (s,

| | |
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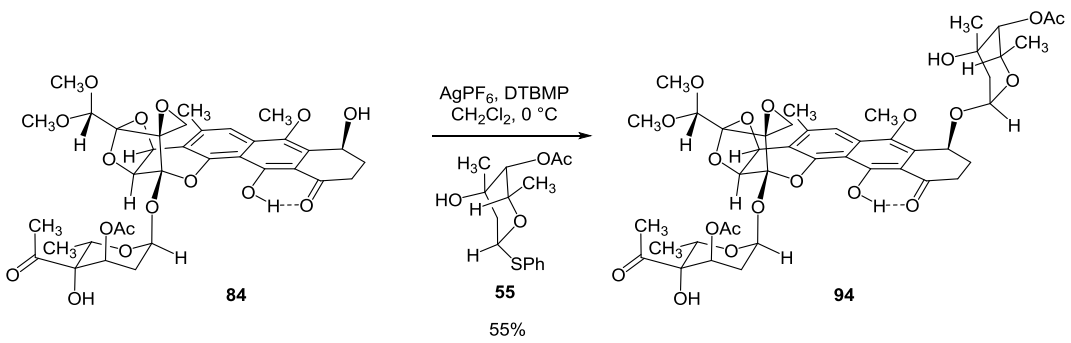
| | 3H), 3.71 (d, 1H, *J* = 9.5 Hz), 3.63 (s, 3H), 3.49 (s, 3H), 3.2 (ddd, 1H, *J* = 18.1, 13.6, 5.2 Hz), 2.97 (d, 1H, *J* = 5.6 Hz), 2.9 (d, 1H, *J* = 5.9 Hz), 2.61 (s, 3H), 2.60–2.51 (m, 2H), 2.49 (s, 3H), 2.43 (dt, 1H, *J* = 14.5, 3.3 Hz), 2.12 (d, 1H, *J* = 14.7 Hz), 2.06 (tdd, 1H, *J* = 13.5, 4.0, 1.6 Hz), 1.09 (d, 3H, *J* = 6.3 Hz). |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 210.5, 204.3, 163.0, 151.6, 143.8, 141.8, 138.2, 135.2, 128.4, 128.3, 127.8, 127.7, 116.7, 114.7, 114.1, 109.7, 104.6, 101.4, 99.7, 94.8, 79.6, 71.5, 70.6, 70.3, 69.3, 68.3, 67.7, 63.8, 62.8, 56.8, 56.1, 48.2, 32.4, 31.5, 27.8, 26.5, 20.3, 14.5. |
| **FTIR**, $cm^{-1}$: (neat) | 3512 (br w), 2926 (m), 1621 (s), 1389 (s), 1084 (s), 984 (s). |
| **HRMS**: (ESI) | Calcd for $(C_{40}H_{44}O_{15}+Na)^+$ 787.2572<br>Found 787.2571 |
| **TLC**: (67% ethyl acetate–hexane) | $R_f$ = 0.32 (UV, CAM) |

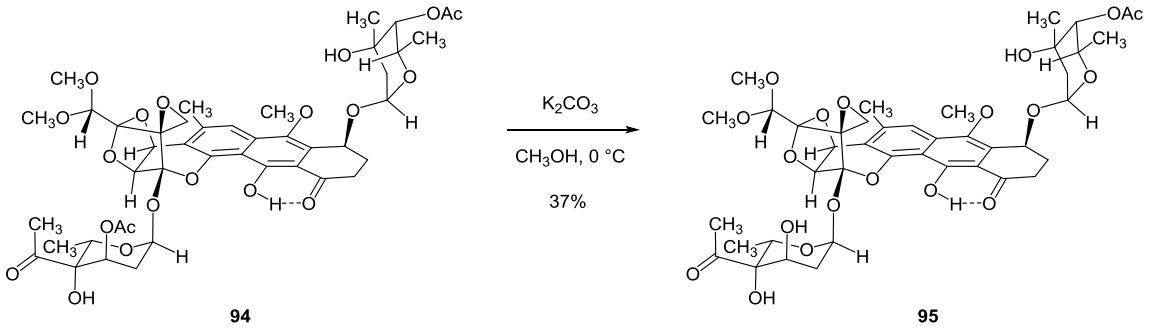



3"-*O*-Acetyl-2-deoxytrioxacarcin A (**94**).

A suspension of benzylic alcohol **84** (9.0 mg, 13 μmol, 1 equiv), 1-phenylthiotrioxacarcinoside A **55** (11 mg, 38 μmol, 3.0 equiv, a 3:1 mixture of α and β anomers, respectively), 2,6-di-*tert*-butyl-4-methylpyridine (21 mg, 0.10 mmol, 8.0 equiv), and powdered 4-Å molecular sieves (~25 mg) in dichloromethane (0.6 mL) was stirred for 20 min at 23 °C. The mixture was cooled to 0 °C in an ice bath. Silver hexafluorophosphate (19 mg, 75 μmol, 6.0 equiv) was added in one portion. After 1 h, a solution of triethylamine (88 μL, 0.63 mmol, 50 equiv) in dichloromethane (10 mL) was added. The suspension was filtered through a pad of Celite, and the filtrate was concentrated. The residue was immediately purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 40→100% acetonitrile in water, flow rate: 15 mL/min) to provide after concentration pure 3"-*O*-acetyl-2-deoxytrioxacarcin A (**94**) as a yellow-green foam (6.2 mg, 55%, in fractions eluting at 16–21 min).

**$^1$H NMR**: (500 MHz, $CDCl_3$) 14.75 (s, 1H), 7.48 (s, 1H), 5.81 (s, 1H), 5.37 (br, 1H), 5.29 (d, 1H, $J$ = 4.1 Hz), 5.25 (m, 1H), 5.19 (d, 1H, $J$ = 4.2 Hz), 5.00 (q, 1H, $J$ = 6.4 Hz), 4.73 (m, 3H), 4.49 (q,

| | | |
|---|---|---|
| | 1H, *J* = 6.2 Hz), 4.07 (br, 1H), 3.90 (br, 1H), 3.84 (s, 3H), 3.63 (s, 3H), 3.47 (s, 3H), 3.01 (ddd, 1H, *J* = 18.5, 13.8, 5.2 Hz), 2.85 (d, 1H, *J* = 5.5 Hz), 2.78 (d, 1H, *J* = 5.5 Hz), 2.66 (d, 1H, *J* = 18.2 Hz), 2.59 (s, 3H), 2.59–2.52 (m, 1H), 2.43 (m, 1H), 2.35 (s, 3H), 2.32 (m, 1H), 2.22 (s, 3H), 2.20 (m, 1H), 2.12 (s, 3H), 1.93 (dd, 1H, *J* = 14.4, 3.8 Hz), 1.60 (d, 1H, *J* = 14.4 Hz), 1.22 (d, 3H, *J* = 6.5 Hz), 1.07 (d, 3H, *J* = 6.8 Hz), 1.06 (s, 3H). | |
| **$^{13}$C NMR**:<br>(125 MHz, $CDCl_3$) | 208.7, 203.1, 170.4, 170.2, 163.3, 151.9, 144.1, 142.3, 135.0, 126.7, 116.6, 114.9, 114.6, 109.2, 104.5, 101.6, 99.4, 96.9, 92.4, 78.2, 74.5, 72.0, 70.7, 69.1, 68.8, 68.3, 66.3, 64.5, 62.8, 62.7, 56.5, 55.9, 47.5, 36.5, 32.4, 28.9, 28.4, 27.0, 25.6, 21.3, 20.9, 20.3, 16.9, 14.5. | |
| **FTIR**, $cm^{-1}$:<br>(neat) | 3480 (br, w), 2926 (m), 1740 (s), 1620 (m), 1373 (s), 1236 (s), 999 (s). | |
| **HRMS**:<br>(ESI) | Calcd for $(C_{44}H_{54}O_{20}+H)^+$<br>Found | 903.3281<br>903.3284 |
| **TLC**:<br>(5% methanol–dichloromethane) | $R_f$ = 0.47 (UV, CAM) | |



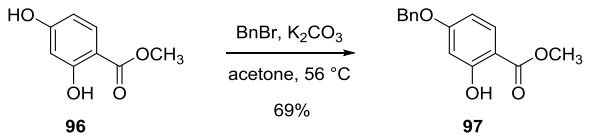

94 95

2-deoxytrioxacarcin A (**95**).

Potassium carbonate (1.8 mg, 13 μmol, 2.0 equiv) was added to an ice-cooled solution of 3"-*O*-Acetyl-2-deoxytrioxacarcin A **94** (6.0 mg, 6.7 μmol, 1 equiv) in methanol (1.3 mL). After 1 h, pH 7 aqueous phosphate buffer solution (10 mL) was added. The mixture was concentrated by rotary evaporation to remove methanol. The concentrated solution was extracted with dichloromethane (3 × 30 mL). The organic layers were combined and the combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 20→80% acetonitrile in water, flow rate: 15 mL/min) to provide after concentration 2-deoxytrioxacarcin A (**95**) (2.1 mg, 37%).

**$^1$H NMR**: (600 MHz, $CDCl_3$) 14.80 (s, 1H), 7.51 (s, 1H), 5.84 (br s, 1H), 5.38 (br s, 1H), 5.35 (d, 1H, $J$ = 4.2 Hz), 5.26 (m, 1H), 5.24 (d, 1H, $J$ = 4.2 Hz), 5.02 (q, 1H, $J$ = 6.4 Hz), 4.78 (s, 1H), 4.74 (s, 1H), 4.49 (q, 1H, $J$ = 6.2 Hz), 4.25 (d, 1H, $J$ = 9.9 Hz), 4.16 (s, 1H), 4.07 (s, 1H), 3.85 (s, 3H), 3.71 (d, 1H, $J$ = 9.7 Hz), 3.63 (s, 3H), 3.49 (s, 3H), 3.03 (ddd, 1H, $J$ =

| | |
|---|---|
| | 19.3, 13.6, 5.1 Hz), 2.99 (d, 1H, *J* = 5.7 Hz), 2.94 (d, 1H, *J* = 5.7 Hz), 2.68 (dd, 1H, *J* = 18.2, 4.4 Hz), 2.61 (s, 3H), 2.50 (s, 3H), 2.46–2.41 (m, 1H), 2.25–2.18 (m, 1H), 2.15–2.11 (m, 1H), 2.13 (s, 3H), 1.94 (dd, 1H, *J* = 14.5, 4.0 Hz), 1.60 (d, 1H, *J* = 14.5 Hz), 1.22 (d, 3H, *J* = 6.6 Hz), 1.09 (d, 3H, *J* = 6.4 Hz), 1.07 (s, 3H). |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 210.4, 203.3, 170.4, 163.3, 151.8, 144.1, 142.2, 135.1, 126.8, 116.9, 114.8, 114.5, 109.3, 104.7, 101.5, 99.7, 97.0, 94.8, 79.6, 74.5, 71.4, 70.2, 69.3, 68.8, 68.3, 66.2, 63.8, 62.8, 62.7, 56.8, 56.2, 48.2, 36.5, 32.4, 31.5, 28.4, 27.8, 25.6, 20.9, 20.3, 16.9, 14.5. |
| **FTIR**, $cm^{-1}$: (neat) | 3509 (br m), 2928 (s), 1719 (s), 1622 (s), 1389 (s), 1234 (s), 1107 (s), 999 (s). |
| **HRMS**: (ESI) | Calcd for $(C_{42}H_{52}O_{19}+Na)^+$ 883.2995<br>Found 883.2974 |
| **TLC**: (5% methanol–dichloromethane) | $R_f$ = 0.41 (UV, CAM) |




**96** **97**

Methyl 4-(Benzyloxy)-2-hydroxybenzoate (**97**).[71]

Benzyl bromide (3.54 mL, 29.7 mmol, 1.00 equiv) was added dropwise to an ice-cooled suspension of methyl 2,4-dihydroxybenzoate (**96**) (5.00 g, 29.7 mmol, 1 equiv) and potassium carbonate (5.75 g, 41.6 mmol, 1.4 equiv) in acetone (30 mL). The suspension was heated to reflux. After 18 h, the mixture was concentrated. The residue was partitioned between ethyl acetate (100 mL) and water (50 mL). The layers were separated. The basic aqueous layer was neutralized (pH ~7) by addition of 1.0 M aqueous hydrochloric acid solution, and the neutralized solution was extracted with ethyl acetate (100 mL). The organic layers were combined. The combined solution was washed sequentially with water (50 mL) then saturated aqueous sodium chloride solution (50 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was recrystallized from methanol to provide methyl 4-(benzyloxy)-2-hydroxybenzoate (**97**) as a white solid (5.31 g, 69%).[71]

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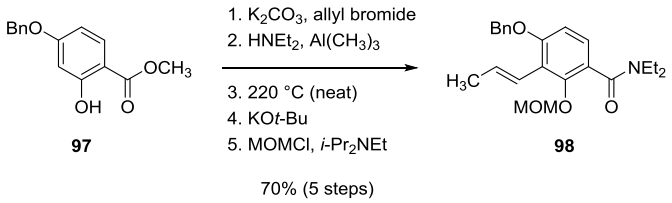
|---|---|
| **$^1$H NMR**: (600 MHz, $CDCl_3$) | 10.96 (s, 1H), 7.75 (d, 1H, *J* = 8.8 Hz), 7.43–7.38 (m, 4H), 7.36–7.32 (m, 1H), 6.53 (d, 1H, *J* = 2.3 Hz), 6.51 (dd, 1H, *J* = 8.8, 2.4 Hz), 5.08 (s, 2H), 3.91 (s, 3H). |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 170.3, 164.6, 163.7, 136.0, 131.2, 128.6, 128.2, 127.5, 108.0, 105.6, 101.6, 70.1, 51.9. |

| | | |
|---|---|---|
| **FTIR**, $cm^{-1}$: | 1667 (s), 1620 (s), 1439 (s), 1346 (s), 1252 (s), 1138 (s). | |
| (neat) | | |
| **HRMS**: | Calcd for $(C_{15}H_{14}O_4+H)^+$ | 259.0965 |
| (ESI) | Found | 259.0970 |




(*E*)-4-(benzyloxy)-*N*,*N*-diethyl-2-(methoxymethoxy)-3-(prop-1-en-1-yl)benzamide (**98**).

Allyl bromide (3.56 mL, 41.1 mmol, 2.0 equiv) was added to a vigorously stirred suspension of methyl 4-(benzyloxy)-2-hydroxybenzoate (**97**) (5.31 g, 20.6 mmol, 1 equiv) and potassium carbonate (5.68 g, 41.1 mmol, 2.0 equiv) in *N*,*N*-dimethylformamide (40 mL) at 23 °C. After 18 h, the mixture was partitioned between ethyl acetate (600 mL) and water (200 mL). The layers were separated. The organic layer was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide methyl 2-(allyloxy)-4-(benzyloxy)benzoate, which was used in the next step without purification.

Trimethylaluminum (1.8 M solution in toluene, 21.7 mL, 39.0 mmol, 1.9 equiv) was added to an ice-cooled solution of diethylamine (8.1 mL, 78 mol, 3.8 equiv) in benzene (10 mL). After 10 min, the cooling bath was removed and the reaction flask was allowed to warm to 23 °C. A solution of methyl 2-(allyloxy)-4-(benzyloxy)benzoate (1 equiv, see paragraph above) in toluene (10 mL) was added to the reaction mixture over 5 min by cannula. The reaction mixture was heated at reflux in an oil bath at 120 °C (CAUTION: gas evolution). After 7 h, the heating bath was removed and the reaction flask was allowed to cool to 23 °C. The reaction mixture was poured carefully into a mixture of ice water (100 mL) and 12 M aqueous hydrochloric acid solution (2 mL). The layers were separated. The aqueous layer was extracted with ethyl acetate (3 × 100 mL). The organic layers were combined. The combined solution was washed sequentially with

water (100 mL) then saturated aqueous sodium chloride solution (50 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide 2-(allyloxy)-4-(benzyloxy)-*N*,*N*-diethylbenzamide, which was used in the next step without purification.

2-(allyloxy)-4-(benzyloxy)-*N*,*N*-diethylbenzamide (see paragraph above) was heated neat in an oil bath at 220 °C. After 5 h, the heating bath was removed and the reaction flask was allowed to cool to 23 °C. The product, 3-allyl-4-(benzyloxy)-*N*,*N*-diethyl-2-hydroxybenzamide, was used in the next step without purification.

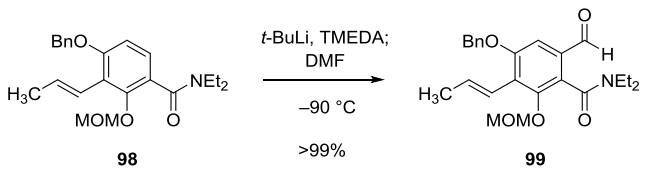
Potassium *tert*-butoxide (11.52 g, 103 mmol, 5.0 equiv) was added to a solution of 3-allyl-4-(benzyloxy)-*N*,*N*-diethyl-2-hydroxybenzamide (1 equiv, see paragraph above) in dimethyl sulfoxide (21 mL) at 23 °C. The reaction flask was heated in an oil bath at 120 °C. After 70 min, the heating bath was removed and the reaction flask was allowed to cool to 23 °C. The reaction mixture was diluted with water (40 mL). The diluted solution was acidified to pH ~2 with 6.0 M aqueous hydrochloric acid solution (~50 mL). The mixture was extracted with ethyl acetate (2 × 100 mL). The organic layers were combined. The combined solution was washed sequentially with water (5 × 100 mL) then saturated aqueous sodium chloride solution (100 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide (*E*)-4-(benzyloxy)-*N*,*N*-diethyl-2-hydroxy-3-(prop-1-en-1-yl)benzamide, which was used in the next step without purification.

Chloromethyl methyl ether (2.34 mL, 30.7 mmol, 1.5 equiv) was added to a solution of (*E*)-4-(benzyloxy)-*N*,*N*-diethyl-2-hydroxy-3-(prop-1-en-1-yl)benzamide (1

equiv, see paragraph above) and *N*,*N*-diisopropylethylamine (7.17 mL, 41.0 mmol, 2.0 equiv) in dichloromethane (84 mL) at 0 °C. After 5 min, the cooling bath was removed and the reaction flask was allowed to warm to 23 °C. After 22 h, the reaction mixture was partitioned between water (200 mL) and dichloromethane (200 mL). The layers were separated. The aqueous layer was extracted with dichloromethane (200 mL). The organic layers were combined. The combined solution was washed with saturated aqueous sodium chloride solution (100 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (20% ethyl acetate–hexanes initially, grading to 40% ethyl acetate–hexanes) to provide (*E*)-4-(benzyloxy)-*N*,*N*-diethyl-2-(methoxymethoxy)-3-(prop-1-en-1-yl)benzamide (**98**) as a yellow oil (5.50 g, 70% over 5 steps).

| | |
|---|---|
| **$^1$H NMR**: (500 MHz, $CDCl_3$) | 7.43–7.32 (m, 5H), 7.02 (d, 1H, $J$ = 8.7 Hz), 6.75 (d, 1H, $J$ = 8.2 Hz), 6.61 (m, 2H), 5.11 (s, 2H), 5.00 (br, 2H), 3.68 (br, 1H), 3.51 (s, 3H), 3.41 (br, 1H), 3.30–3.12 (br, 2H), 1.90 (d, 3H, $J$ = 5.0 Hz), 1.24 (t, 3H, $J$ = 6.9 Hz), 1.04 (t, 3H, $J$ = 6.9 Hz). |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 168.7, 157.3, 151.0, 136.4, 131.8, 128.2, 127.6, 126.9, 125.4, 124.8, 121.1, 120.9, 108.3, 99.4, 70.3, 57.3, 42.8, 38.8, 19.7, 13.6, 12.6. |

| | | |
|---|---|---|
| **FTIR**, $cm^{-1}$: | 2972 (w), 1626 (s), 1427 (m), 1265 (m), 1265 (m), 1055 | |
| (neat) | (s), 926 (s), 733 (s). | |
| **HRMS**: | Calcd for $(C_{23}H_{29}NO_4+Na)^+$ | 406.1998 |
| (ESI) | Found | 406.1989 |
| **TLC**: | $R_f$ = 0.41 (UV, CAM) | |
| (50% ethyl acetate–hexanes) | | |



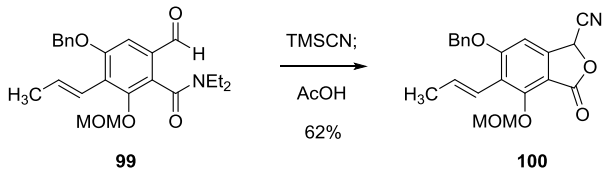

**98** **99**

Aldehyde **99**.

*tert*-Butyllithium (1.7 M solution in pentane, 1.84 mL, 3.13 mmol, 1.2 equiv) was added dropwise to a solution of *N*,*N*,*N*,*N*-tetramethylethylenediamine (472 μL, 3.13 mmol, 1.2 equiv) and (*E*)-4-(benzyloxy)-*N*,*N*-diethyl-2-(methoxymethoxy)-3-(prop-1-en-1-yl)benzamide (**98**) (1.00 g, 2.61 mmol, 1 equiv) in tetrahydrofuran (26 mL) at –90 °C in a liquid nitrogen–hexane bath. After 5 min, dimethylformamide (2.42 mL, 31.3 mmol, 12 equiv) was added. After 10 min, the cooling bath was removed and the reaction flask was allowed to warm to 23 °C. After 40 min, the reaction mixture was diluted with water (10 mL), and the diluted solution was partially concentrated to remove the volatile organic solvents. The aqueous residue was extracted with ethyl acetate (3 × 50 mL). The organic layers were combined. The combined solution was washed with water (2 × 50 mL) then saturated aqueous sodium chloride solution (50 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide aldehyde **99** as a pale yellow oil (1.07 g, >99%).

**$^1$H NMR**: (500 MHz, $CDCl_3$) 9.90 (s, 1H), 7.45–7.36 (m, 5H), 7.34 (s, 1H), 6.83 (dq, 1H, *J* = 16.0, 6.9 Hz), 6.64 (dd, 1H, *J* = 16.0, 1.8 Hz), 5.17 (s, 2H), 5.06 (d, 1H, *J* = 5.0 Hz), 5.01 (d, 1H, *J* = 5.0 Hz), 3.68–3.56 (m, 2H), 3.54 (s, 3H), 3.21–3.11 (m, 2H), 1.93 (dd, 3H, *J* = 6.9, 1.8 Hz), 1.30 (t, 3H, *J* = 7.3

| | | |
|---|---|---|
| | Hz), 1.04 (t, 3H, $J$ = 7.3 Hz). | |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 189.5, 165.9, 157.4, 151.3, 136.0, 135.6, 131.2, 128.6, 128.5, 128.1, 127.6, 127.4, 121.2, 107.0, 100.1, 70.9, 57.9, 43.3, 39.3, 20.2, 13.8, 12.8. | |
| **FTIR**, $cm^{-1}$: (neat) | 2936 (w), 1694 (m), 1628 (s), 1288 (m), 924 (m). | |
| **HRMS**: (ESI) | Calcd for $(C_{24}H_{29}NO_5+Na)^+$ | 434.1938 |
| | Found | 434.1942 |
| **TLC**: (50% ethyl acetate–hexanes) | $R_f$ = 0.47 (UV, CAM) | |



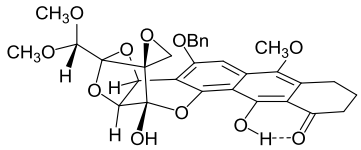

**99** **100**

Cyanophthalide **100**.

Cyanotrimethylsilane (524 μL, 3.91 mmol, 1.5 equiv) was added to an ice-cooled solution of aldehyde **99** (1.07 g, 2.61 mmol, 1 equiv) in dichloromethane (5.2 mL). After 5 min, potassium cyanide (1.7 mg, 0.026 mmol, 0.01 equiv) and 18-crown-6 (6.9 mg, 0.026 mmol, 1 equiv) were added. After 5 min, the cooling bath was removed and the reaction flask was allowed to warm to 23 °C. After 5 h, the solution was concentrated carefully. The residue was dissolved in glacial acetic acid (5.2 mL) (CAUTION: the highly toxic gas hydrogen cyanide may be generated upon treatment of the residue with acetic acid: This operation should be conducted in a well-ventilated fume hood). The resulting solution was stirred at 23 °C. After 41 h, the solvent was removed by rotary evaporation. The residue was partitioned between saturated aqueous sodium bicarbonate solution (50 mL) and ethyl acetate (50 mL). The layers were separated. The organic layer was washed with water (50 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (10% ethyl acetate–hexanes initially, grading to 30% ethyl acetate–hexanes) to provide cyanophthalide **100** as a white foam (588 mg, 62% over two steps).

**$^1$H NMR**: (500 MHz, $CDCl_3$) 7.44–7.33 (m, 5H), 6.89 (s, 1H), 6.64 (dq, 1H, $J$ = 16.1, 6.2 Hz), 6.57 (d, 1H, $J$ = 16.1 Hz), 5.90 (s, 1H), 5.34 (q,

2H, $J$ = 6.5 Hz), 5.27 (d, 1H, $J$ = 11.7 Hz), 5.20 (d, 1H, $J$ = 11.7 Hz), 3.56 (s, 3H), 1.93 (d, 3H, $J$ = 5.9 Hz).



**101**

Benzyl ether **101.**

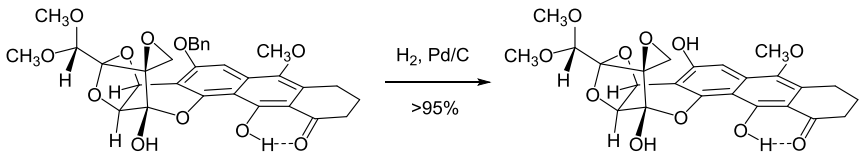
Benzyl ether **101** was assembled from the epoxy diazo diketone **18**, cyanophthalide **100**, and cyclohexenone by an analogous sequence to that used for the synthesis of synthetic precursor **23** (see Scheme 1.3).

| | |
|---|---|
| **$^1$H NMR**: (500 MHz, $CDCl_3$) | 14.97 (s, 1H), 7.48 (d, 2H, *J* = 7.0 Hz), 7.40–7.31 (m, 3H), 7.00 (s, 1H), 5.55 (d, 1H, *J* = 4.0 Hz), 5.30 (d, 1H, *J* = 12.1 Hz), 5.23 (d, 1H, *J* = 12.1 Hz), 4.78 (d, 1H, *J* = 4.0 Hz), 4.72 (s, 1H), 4.39 (br s, 1H), 3.66 (s, 3H), 3.60 (s, 3H), 3.48 (s, 3H), 3.20 (d, 1H, *J* = 5.5 Hz), 3.09 (d, 1H, *J* = 5.5 Hz), 3.01 (t, 2H, *J* = 6.2 Hz), 2.70 (m, 2H), 2.07 (m, 2H). |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 204.0, 163.0, 159.2, 152.8, 142.0, 136.5, 136.2, 130.7, 128.6, 128.1, 127.3, 110.1, 109.6, 106.2, 103.8, 100.0, 98.7, 95.3, 72.8, 70.4, 69.5, 67.2, 60.4, 56.7, 56.2, 50.6, 38.6, 23.6, 22.1. |
| **FTIR**, $cm^{-1}$: (neat) | 3343 (br w), 2945 (w), 1616 (s), 13897 (m), 1080 (s). |

| | | |
|---|---|---|
| **HRMS**: | Calcd for $(C_{31}H_{30}O_{11}+H)^+$ | 579.1861 |
| (ESI) | Found | 579.1856 |

**TLC**: $R_f$ = 0.13 (UV, CAM)

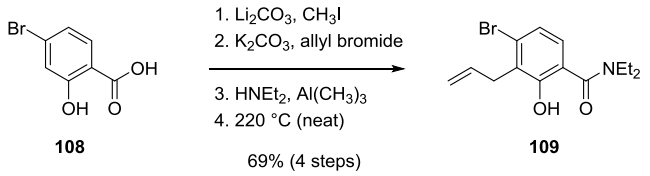
(40% ethyl acetate–hexanes)




Phenol **102**.

Palladium on activated charcoal (10 wt. %, 1.1 mg, 1.0 μmol, 0.1 equiv) was added to a solution of benzyl ether **101** (6 mg, 10 μmol, 1 equiv) in ethanol (200 μL) at 23 °C. The mixture was saturated with hydrogen by bubbling hydrogen gas below the liquid surface for 2 min through a 22-gague stainless steel needle, and the mixture was subsequently stirred under a hydrogen atmosphere at 23 °C. After 2 h, the mixture was diluted with ethyl acetate (20 mL) filtered through a short pad of Celite. The filtrate was concentrated to provide phenol **102** (5 mg, >95%) as a yellow-green oil. During attempts at purification by rp-HPLC and upon standing in chloroform, phenol **102** underwent decomposition, forming a complex mixture of products.

| | | |
|---|---|---|
| **$^1$H NMR**: (500 MHz, $CDCl_3$) | 16.62 (br s, 1H), 10.67 (s, 1H), 6.91 (s, 1H), 5.56 (d, 1H, $J$ = 4.0 Hz), 4.79 (d, 1H, $J$ = 3.7 Hz), 4.69 (s, 1H), 4.29 (br s, 1H), 3.75 (s, 3H), 3.58 (s, 3H), 3.48 (s, 3H), 3.26 (d, 1H, $J$ = 5.1 Hz), 3.05 (d, 1H, $J$ = 5.1 Hz), 3.04–2.92 (m, 2H), 2.73 (t, 2H, $J$ = 6.4 Hz), 2.09 (quin, 2H, $J$ = 6.2 Hz). | |
| **HRMS**: (ESI) | Calcd for $(C_{24}H_{24}O_{11}+Na)^+$ | 511.1211 |
| | Found | 511.1227 |




**108** **109**

3-allyl-4-bromo-*N*,*N*-diethyl-2-hydroxybenzamide (**109**).

Iodomethane (1.66 mL, 26.6 mmol, 1.1 equiv) was added to a mixture of 4-bromo-2-hydroxybenzoic acid (**108**) (5.24 g, 24.2 mmol, 1 equiv) and lithium carbonate (1.96 g, 26.6 mmol, 1.1 equiv) in dimethylformamide at 23 °C. The reaction flask was heated in an oil bath at 60 °C. After 3.5 h, the warm mixture was partitioned between ice water (50 mL) and ethyl acetate (80 mL). The layers were separated. The aqueous layer was extracted with ethyl acetate (80 mL). The organic layers were combined. The combined solution was washed sequentially with water (2 × 50 mL) then saturated aqueous sodium chloride solution (50 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the product, methyl 4-bromo-2-hydroxybenzoate, which was used in the next step without purification.

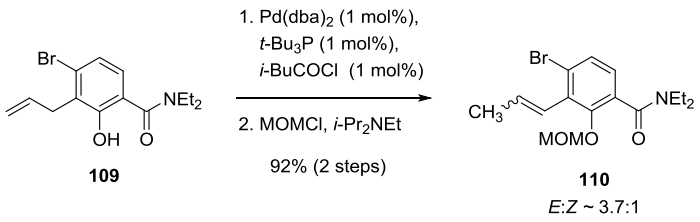
Methyl 4-bromo-2-hydroxybenzoate (1 equiv, see paragraph above) was added to an ice-cooled suspension of sodium hydride (60% dispersion in mineral oil, 1.16 g, 29.0 mmol, 1.2 equiv) and allyl bromide (4.18 mL, 48.3 mmol, 2.0 equiv) in dimethylformamide (40 mL). After 10 min, the cooling bath was removed and the reaction flask was allowed to warm to 23 °C. After 22 h, the mixture was partitioned carefully between ice water (50 mL) and ether (50 mL). The layers were separated. The aqueous layer was extracted with ether (2 × 50 mL). The organic layers were combined. The combined solution was washed successively with water (3 × 40 mL) then saturated aqueous sodium chloride solution (40 mL) and the washed solution was dried over

sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the product, methyl 2-(allyloxy)-4-bromobenzoate, which was used in the next step without purification.

Trimethylaluminum (2.0 M solution in toluene, 23.0 mL, 45.9 mmol, 1.9 equiv) was added to an ice-cooled solution of diethylamine (9.59 mL, 92.0 mmol, 3.8 equiv) in benzene (15 mL). After 10 min, the cooling bath was removed and the reaction flask was allowed to warm to 23 °C. A solution of methyl 2-(allyloxy)-4-bromobenzoate (1 equiv, see paragraph above) in toluene (15 mL) was added. The reaction flask was heated in an oil bath at 120 °C (CAUTION: gas evolution). After 2.5 h, the oil bath was removed and the reaction flask was allowed to cool to 23 °C. The solution was poured carefully into 0.2 M aqueous hydrochloric acid solution (100 mL). The mixture was extracted with ethyl acetate (3 × 100 mL). The organic layers were combined. The combined solution was washed sequentially with water (100 mL) then saturated aqueous sodium chloride solution (50 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the product, 2-(allyloxy)-4-bromo-*N*,*N*-diethylbenzamide, which was used in the next step without purification.

2-(allyloxy)-4-bromo-*N*,*N*-diethylbenzamide (see paragraph above) was heated neat in an oil bath at 220 °C. After 4 h, the heating bath was removed and the reaction flask was allowed to cool to 23 °C. The product was purified by flash-column chromatography (5% ethyl acetate–hexanes initially, grading to 15% ethyl acetate–hexanes) to provide 3-allyl-4-bromo-*N*,*N*-diethyl-2-hydroxybenzamide (**109**) as a yellow oil (5.20 g, 69% yield over four steps).

| | | |
|---|---|---|
| **$^{1}$H NMR**: (600 MHz, $CDCl_3$) | 10.4 (br s, 1H), 7.07 (d, 1H, $J$ = 8.2 Hz), 7.01 (d, 1H, $J$ = 8.8 Hz), 5.96 (dd, 1H, $J$ = 17.0, 10.6 Hz), 5.10–5.04 (m, 2H), 3.62 (d, 2H, $J$ = 6.5 Hz), 3.5 (q, 4H, $J$ = 7.2 Hz), 1.27 (t, 6H, $J$ = 7.0 Hz). | |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 171.2, 157.6, 134.3, 128.8, 128.4, 125.8, 122.3, 116.6, 115.5, 42.2, 33.8, 13.2. | |
| **FTIR**, $cm^{-1}$: (neat) | 2974 (m), 2932 (m), 1611 (s), 1587 (s), 1452 (s), 1275 (s). | |
| **HRMS**: (ESI) | Calcd for $(C_{14}H_{18}NO_2Br+H)^+$ | 312.0594 |
| | Found | 312.0575 |
| **TLC**: (20% ethyl acetate–hexanes) | $R_f$ = 0.49 (UV) | |



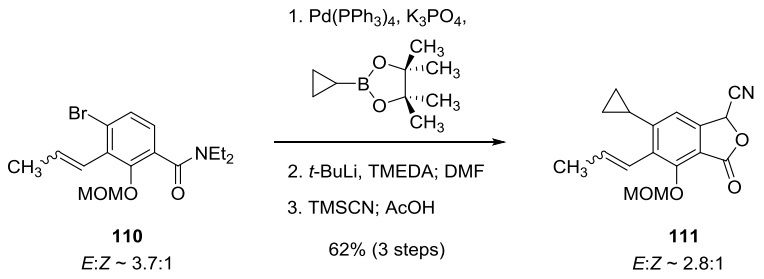

4-bromo-*N*,*N*-diethyl-2-(methoxymethoxy)-3-(prop-1-en-1-yl)benzamide (**110**).

Bis(dibenzylideneacetone)palladium(0) (92 mg, 0.16 mmol, 0.01 equiv), tri-*tert*-butylphosphine (39 μL, 0.16 mmol, 0.01 equiv), and isobutyryl chloride (17 μL, 0.16 mmol, 0.01 equiv)[77] were added sequentially to a solution of 3-allyl-4-bromo-*N*,*N*-diethyl-2-hydroxybenzamide (**109**) (5.00 g, 16.0 mmol, 1 equiv) in toluene (40 mL) at 23 °C. The flask was heated in an oil bath at 80 °C. After 23 h, the solution was concentrated. The residue (1 equiv, see above) was dissolved in dichloromethane (80 mL). *N*,*N*-diisopropylethylamine (7.05 mL, 40.4 mmol, 2.5 equiv) and chloromethyl methyl ether (2.45 mL, 32.3 mmol, 2.0 equiv) were added at 23 °C. After 20 h, the mixture was partitioned between water (100 mL) and dichloromethane (150 mL). The layers were separated. The aqueous layer was extracted with dichloromethane (150 mL). The organic layers were combined. The combined solution was washed sequentially with water (100 mL) the saturated aqueous sodium chloride solution (100 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (10% ethyl acetate–hexanes initially, grading to 40% ethyl acetate–hexanes) to provide the product, 4-bromo-*N*,*N*-diethyl-2-(methoxymethoxy)-3-(prop-1-en-1-yl)benzamide (**110**) as a ~3.7:1 mixture of *E*- and *Z*-isomers, respectively (the alkene stereochemistry is inconsequential) (5.25 g, 92% over two steps).

| | | |
|---|---|---|
| **$^{1}$H NMR**: (500 MHz, $CDCl_3$) | ***E*-isomer (major):** 7.38 (d, 1H, $J$ = 8.2 Hz), 6.94 (d, 1H, $J$ = 8.2 Hz), 6.42–6.31 (m, 2H), 5.04–4.90 (m, 2H), 3.65 (br m, 1H), 3.47 (s, 3H), 3.44 (br m, 1H), 3.18 (br m, 2H), 1.93 (d, 3H, $J$ = 6.0 Hz), 1.24 (t, 3H, $J$ = 7.1 Hz), 1.06 (t, 3H, $J$ = 7.1 Hz). | |
| | ***Z*-isomer (minor):** 7.41 (d, 1H, $J$ = 8.2 Hz), 7.03 (d, 1H, $J$ = 8.2 Hz), 6.28 (dd, 1H, $J$ = 11.2, 1.1 Hz), 5.95 (dq, 1H, $J$ = 11.4, 6.9 Hz), 5.04–4.90 (m, 2H), 3.65 (br m, 1H), 3.46 (s, 3H), 3.44 (br m, 1H), 3.18 (br m, 2H), 1.58 (d, 3H, $J$ = 6.9, 1.8 Hz), 1.20 (t, 3H, $J$ = 7.1 Hz), 1.06 (t, 3H, $J$ = 7.1 Hz). | |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 167.9, 167.8, 151.2, 151.1, 134.0, 133.9, 132.9, 131.7, 130.2, 129.0, 128.4, 127.1, 126.1, 125.4, 125.2, 125.1, 124.5, 99.6, 99.5, 98.1, 57.6, 57.4, 43.0, 42.9, 39.0, 39.0, 19.1, 19.1, 15.1, 14.9, 13.8, 12.8. | |
| **FTIR**, $cm^{-1}$: (neat) | 2976 (w), 1632 (s), 1429 (s), 1159 (s), 949 (s). | |
| **HRMS**: (ESI) | Calcd for $(C_{16}H_{22}NO_3Br+H)^+$ | 356.0856 |
| | Found | 356.0863 |

**TLC**: $R_f$ = 0.19, 0.13 (UV, CAM)

(20% ethyl acetate–hexanes)




Cyanophthalide **111**.

In a 150-mL heavy-walled glass pressure vessel, a suspension of 4-bromo-*N*,*N*-diethyl-2-(methoxymethoxy)-3-(prop-1-en-1-yl)benzamide (**110**) (940 mg, 2.64 mmol, 1 equiv, a ~3.7:1 mixture of *E*- and *Z*-isomers, respectively), cyclopropylboronic acid pinacol ester (893 μL, 5.28 mmol, 2.0 equiv), and tripotassium phosphate (1.68 g, 7.92 mmol, 3.0 equiv) in a mixture of tetrahydrofuran (17 mL) and water (17 mL) was degassed by sparging for 30 min with a slow stream of argon gas through a 22-gauge stainless steel needle. Tetrakis(triphenylphosphine)palladium(0) (152 mg, 0.132 mmol, 0.05 equiv) was added. The flask was sealed and heated in an oil bath at 120 °C. After 90 min, the heating bath was removed and the reaction flask was allowed to cool to 23 °C. Dichloromethane (250 mL) and saturated aqueous ammonium chloride solution (100 mL) were added. The layers were separated. The organic layer was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (10% ethyl acetate–hexanes initially, grading to 30% ethyl acetate–hexanes) to provide the product, 4-cyclopropyl-*N*,*N*-diethyl-2-(methoxymethoxy)-3-(prop-1-en-1-yl)benzamide.

*tert*-Butyllithium (1.6 M solution in pentane, 3.30 mL, 5.28 mmol, 2.0 equiv) was added dropwise to a solution of *N*,*N*,*N*,*N*-tetramethylethylenediamine (797 μL, 5.28 mmol, 2.0 equiv) and 4-cyclopropyl-*N*,*N*-diethyl-2-(methoxymethoxy)-3-(prop-1-en-1-

yl)benzamide (1 equiv, see paragraph above) in tetrahydrofuran (26 mL) at –78 °C. After 10 min, dimethylformamide (2.42 mL, 31.3 mmol, 12 equiv) was added. After 5 min, the cooling bath was removed and the reaction flask was allowed to warm to 23 °C. After 30 min, the reaction mixture was diluted with water (10 mL), and the diluted solution was partially concentrated to remove the volatile organic solvents. The aqueous residue was extracted with ethyl acetate (3 × 50 mL). The organic layers were combined. The combined solution was washed with water (2 × 50 mL) then saturated aqueous sodium chloride solution (50 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue (1 equiv, see above) was dissolved in dichloromethane (5.3 mL). Cyanotrimethylsilane (531 μL, 3.96 mmol, 1.5 equiv) was added at 23 °C. After 5 min, potassium cyanide (1.7 mg, 0.026 mmol, 0.01 equiv) and 18-crown-6 (7 mg, 0.03 mmol, 1 equiv) were added. After 5 min, the cooling bath was removed and the reaction flask was allowed to warm to 23 °C. After 3 h, the solution was concentrated carefully. The residue was dissolved in glacial acetic acid (5.3 mL) (CAUTION: the highly toxic gas hydrogen cyanide may be generated upon treatment of the residue with acetic acid: This operation should be conducted in a well-ventilated fume hood). The resulting solution was stirred at 23 °C. After 71 h, the solvent was removed by rotary evaporation. The residue was partitioned between saturated aqueous sodium bicarbonate solution (50 mL) and ethyl acetate (100 mL). The layers were separated. The organic layer was washed with saturated aqueous sodium chloride solution (50 mL) and the washed solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (10% ethyl acetate–hexanes initially, grading to 25% ethyl

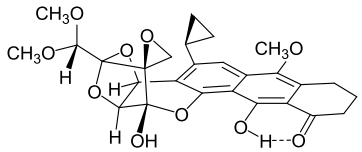
acetate–hexanes) to provide cyanophthalide **111** as a colorless oil (490 mg, 62% over three steps, a ~2.8:1 mixture of *E*- and *Z*-isomers, respectively).

| | |
|---|---|
| **$^1$H NMR**: (500 MHz, $CDCl_3$) | ***E*-isomer (major):** 6.88 (s, 1H), 6.54 (dd, 1H, *J* = 16.1, 1.5 Hz), 6.22 (dq, 1H, *J* = 16.1, 6.5 Hz), 5.91 (s, 1H), 5.33–5.28 (m, 2H), 3.55 (s, 3H), 2.20–2.13 (m, 1H), 1.98 (dd, 3H, *J* = 6.6, 1.7 Hz), 1.17–1.10 (m, 2H), 0.86–0.75 (m, 2H).<br><br>***Z*-isomer (minor):** 6.80 (s, 1H), 6.40 (d, 1H, *J* = 6.4 Hz), 6.08 (dq, 1H, *J* = 11.2, 6.8 Hz), 5.92 (s, 1H), 5.33–5.28 (m, 2H), 3.55 (s, 3H), 2.20–2.13 (m, 1H), 1.58 (dd, 3H, *J* = 6.8, 1.5 Hz), 1.17–1.10 (m, 2H), 0.86–0.75 (m, 2H). |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 165.7, 165.6, 154.5, 154.2, 154.1, 153.2, 142.2, 141.3, 135.4, 134.4, 134.1, 131.2, 123.1, 123.0, 114.2, 114.1, 113.5, 112.6, 112.3, 112.1, 100.8, 100.4, 64.7, 64.7, 57.8, 57.6, 19.3, 15.1, 15.0, 14.2, 10.6, 10.6, 10.1, 10.0. |
| **FTIR**, $cm^{-1}$: (neat) | 2934 (w), 1778 (s), 1605 (m), 1157 (m), 991 (s), 912 (s). |
| **HRMS**: | Calcd for $(C_{17}H_{17}NO_4+Na)^+$ 322.1050 |

| | | |
|---|---|---|
| (ESI) | Found | 322.1053 |

**TLC**: $R_f$ = 0.53 (UV, CAM)

(30% ethyl acetate–hexanes)



**112**

Cyclopropyl Analog **112**.

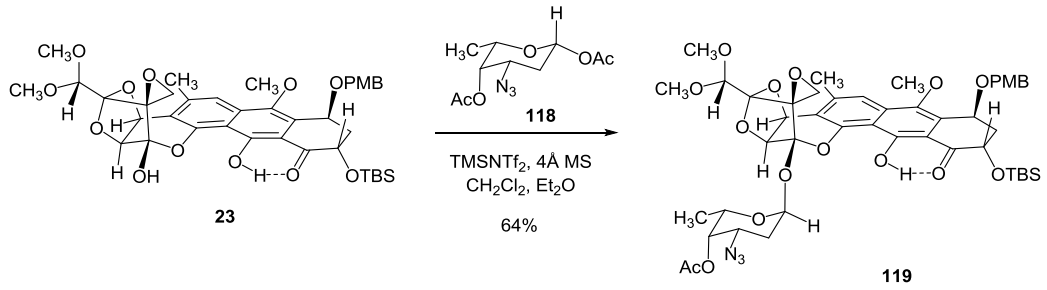
Cyclopropyl analog **112** was assembled from the epoxy diazo diketone **18**, cyanophthalide **111**, and cyclohexenone by an analogous sequence to that used for the synthesis of synthetic precursor **23** (see Scheme 1.3).

| | |
|---|---|
| **$^1$H NMR**: (500 MHz, $CDCl_3$) | 14.92 (br s, 1H), 7.25 (s, 1H), 5.64 (d, 1H, *J* = 4.0 Hz), 4.84 (d, 1H, *J* = 4.0 Hz), 4.72 (s, 1H), 4.38 (br s, 1H), 3.76 (s, 3H), 3.62 (s, 3H), 3.47 (s, 3H), 3.17 (d, 1H, *J* = 5.5 Hz), 3.07 (d, 1H, *J* = 5.1 Hz), 3.03 (td, 2H, *J* = 6.0, 2.9 Hz), 2.74–2.70 (m, 2H), 2.27–2.21 (m, 1H), 2.08 (quin, 2H, *J* = 6.3 Hz), 1.20–1.11 (m, 2H), 1.06–1.01 (m, 1H), 0.57–0.53 (m, 1H). |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 204.4, 162.7, 151.3, 146.7, 142.5, 135.3, 130.1, 114.2, 112.9, 111.2, 111.0, 103.9, 100.1, 98.4, 73.3, 69.4, 69.3, 60.8, 57.0, 56.4, 50.4, 38.7, 23.5, 22.0, 13.5, 9.9, 6.2. |
| **FTIR**, $cm^{-1}$: (neat) | 3420 (br w), 2928 (s), 1717 (m), 1618 (s), 1520 (s), 1366 (s), 1107 (s). |

| | | |
|---|---|---|
| **HRMS**: | Calcd for $(C_{27}H_{28}O_{10}+Na)^+$ | 535.1575 |
| (ESI) | Found | 535.1573 |

**TLC**: $R_f$ = 0.39 (UV, CAM)

(60% ethyl acetate–hexanes)

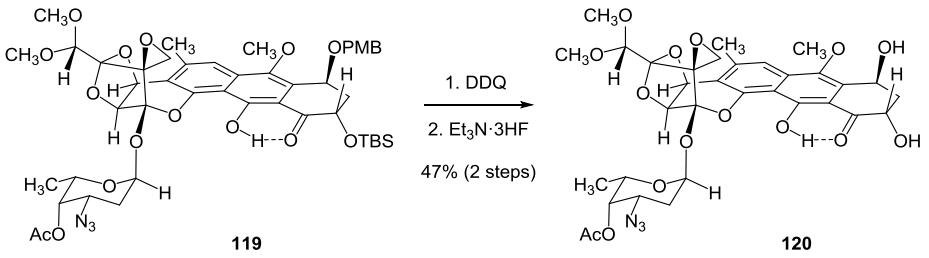



α-Glycoside **119**.

1-*O*-acetyl glycoside **118**[76] (27.3 mg, 0.106 mmol, 16.0 equiv) was added to a solution of synthetic precursor **23** (5.0 mg, 6.6 μmol, 1 equiv) in benzene (0.5 mL). The solution was concentrated and the residue was blanketed with argon. Dichloromethane (100 μL) and ether (30 μL) were added, followed by crushed 4-Å molecular sieves (20 mg). The suspension was stirred for 30 min at 23 °C then was cooled to –78 °C. A freshly prepared solution of *N*-(trimethylsilyl)-bis(trifluoromethanesulfonyl)imide in dichloromethane (1.0 M, 70.4 μL, 0.070 mmol, 10.6 equiv) was added dropwise by syringe over 1.5 h. After 4 h, triethylamine (0.05 mL) was added. The mixture was diluted with 15% methanol–dichloromethane (50 mL) and saturated aqueous sodium bicarbonate solution (10 mL). The layers were separated. The aqueous layer was extracted with dichloromethane (2 × 20 mL). The organic layers were combined and the combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 40→90% acetonitrile in water, flow rate: 15 mL/min) to provide after concentration the pure α-glycoside **119** (4.5 mg, 64%).

[76] 1-*O*-Acetyl glycoside **118** was prepared from daunosamine hydrochloride by a two-step sequence which has been described previously: Zhang, G.; Fang, L.; Zhu, L.; Aimiuwu , J. E.; Shen, J.; Cheng, H.; Muller , M. T.; Lee, G. E.; Sun, D.; Wang, P. G. *J. Med. Chem.* **2005**, *48*, 5269–5278.

| | | |
|---|---|---|
| **$^1$H NMR**: | 14.61 (br, 1H), 7.48 (s, 1H), 7.28 (d, 2H, $J$ = 8.5 Hz), | |
| (600 MHz, $CDCl_3$) | 6.87 (d, 2H, $J$ = 8.7 Hz), 5.91 (t, 1H, $J$ = 2.5 Hz), 5.23 (d, 1H, $J$ = 2.3 Hz), 5.22 (d, 1H, $J$ = 4.1 Hz), 5.19 (m, 2H), 4.97 (dd, 1H, $J$ = 12.6, 5.3 Hz), 4.74 (s, 1H), 4.70 (d, 1H, $J$ = 10.8 Hz), 4.60 (d, 1H, $J$ = 11.0 Hz), 4.40 (q, 1H, $J$ = 6.6 Hz), 4.09 (ddd, 1H, $J$ = 11.2, 6.4, 3.0 Hz), 3.83 (s, 3H), 3.80 (s, 3H), 3.63 (s, 3H), 3.46 (s, 3H), 2.85 (d, 1H, $J$ = 6.0 Hz), 2.74 (d, 1H, $J$ = 6.0 Hz), 2.72 (m, 1H), 2.59 (s, 3H), 2.19 (s, 3H), 2.18–2.15 (m, 2H), 1.15 (d, 3H, $J$ = 6.4 Hz), 0.98 (s, 9H), 0.25 (s, 3H), 0.17 (s, 3H). | |
| **FTIR**, $cm^{-1}$: | 2926 (s), 2854 (m), 2104 (m), 1746 (m), 1620 (s), 1516 | |
| (neat) | (m), 1250 (s), 1107 (s). | |
| **HRMS**: | Calcd for $(C_{47}H_{60}O_{16}N_3Si+H)^+$ | 950.3737 |
| (ESI) | Found | 950.3711 |
| TLC: | $R_f$ = 0.43 (UV, CAM) | |
| (50% ethyl acetate–hexanes) | | |

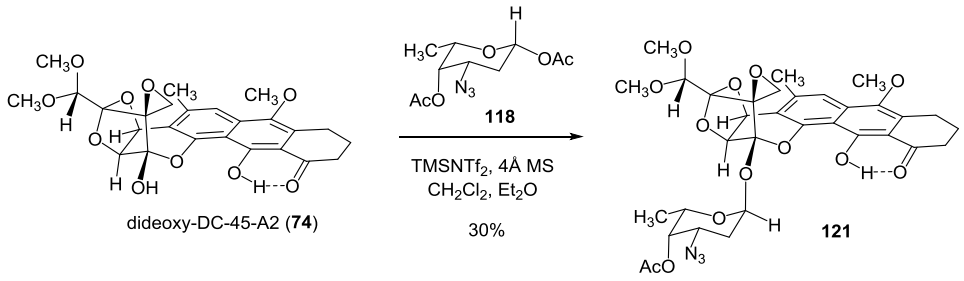



119 120

Azido Diol **120**.

2,3-dichloro-5,6-dicyanobenzoquinone (1.1 mg, 4.7 μmol, 1.0 equiv) was added to a solution of α-glycoside **119** (4.5 mg, 4.7 μmol, 1 equiv) in a mixture of dichloromethane (833 μL) and water (167 μL) at 23 °C. The flask was covered with aluminum foil to exclude light. Over the course of 6.5 h, the color of the reaction mixture changed from myrtle green to yellow. The product solution was partitioned between water (10 mL) and dichloromethane (50 mL). The layers were separated. The aqueous layer was extracted with dichloromethane (3 × 20 mL). The organic layers were combined and the combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was dissolved in acetonitrile (0.5 mL), and triethylamine–trihydrofluoride (23 μL, 0.14 mmol, 30 equiv) was added at 23 °C. The reaction flask was covered with aluminum foil to exclude light. After 14 h, saturated aqueous sodium bicarbonate solution (10 mL) was added. The mixture was extracted with dichloromethane (2 × 50 mL). The organic layers were combined. The combined solution was dried over sodium sulfate. The dried solution was filtered and filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 20→80% acetonitrile in water, flow rate: 15 mL/min) to provide after concentration the pure azido diol **120** (1.6 mg, 47% over 2 steps).

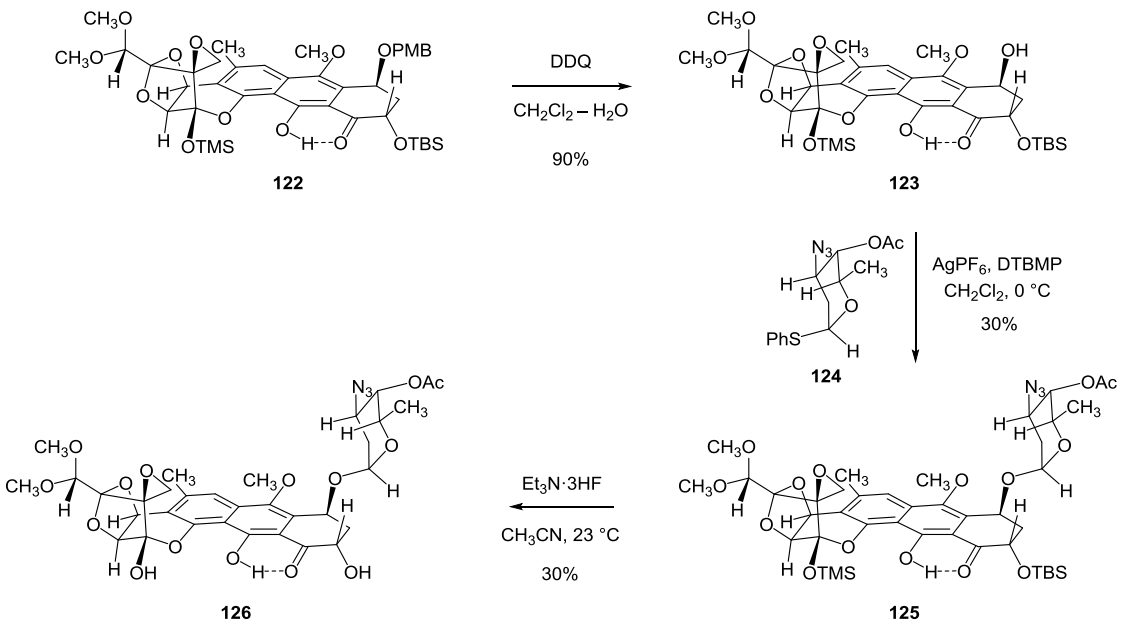
| | | |
|---|---|---|
| **$^1$H NMR**: (600 MHz, $CDCl_3$) | 13.86 (s, 1H), 7.47 (s, 1H), 5.88 (t, 1H, *J* = 2.5 Hz), 5.47 (br, 1H), 5.24 (app d, 2H, *J* = 4.1 Hz), 5.20 (d, 1H, *J* = 4.1 Hz), 4.94 (dd, 1H, *J* = 12.8, 5.5 Hz), 4.75 (s, 1H), 4.41 (q, 1H, *J* = 6.4 Hz), 4.11 (ddd, 1H, *J* = 11.7, 5.7, 3.0 Hz), 3.93 (s, 3H), 3.63 (s, 3H), 3.56 (br s, 1H), 3.47 (s, 3H), 2.89 (d, 1H, *J* = 6.0 Hz), 2.79 (d, 1H, *J* = 6.0 Hz), 2.75 (ddd, 1H, *J* = 13.3, 5.5, 2.8 Hz), 2.61 (s, 3H), 2.21 (br, 1H), 2.20–2.17 (m, 2H), 2.19 (s, 3H), 1.16 (d, 3H, *J* = 6.4 Hz). | |
| **HRMS**: (ESI) | Calcd for $(C_{33}H_{37}O_{15}N_3+H)^+$ | 716.2297 |
| | Found | 716.2278 |




α-Glycoside **121**.

1-*O*-acetyl glycoside **118**[76] (34.9 mg, 0.136 mmol, 6.0 equiv) was added to a solution of dideoxy-DC-45-A2 (**74**)[18] (11.0 mg, 0.023 mmol, 1 equiv) in benzene (2.0 mL). The solution was concentrated and the residue was blanketed with argon. Dichloromethane (850 μL) and ether (280 μL) were added, followed by crushed 4-Å molecular sieves (20 mg). The suspension was stirred for 20 min at 23 °C then was cooled to –78 °C. *N*-(trimethylsilyl)-bis(trifluoromethanesulfonyl)imide (22 μL, 0.090 mmol, 4.0 equiv) was added dropwise by syringe over 10 min. After 2 h, the mixture was diluted with 15% methanol–dichloromethane (50 mL). Saturated aqueous sodium bicarbonate solution (10 mL) was added. The layers were separated. The aqueous layer was extracted with dichloromethane (20 mL). The organic layers were combined and the combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography on silica gel deactivated with triethylamine (40% ethyl acetate–hexanes initially, grading to 50% ethyl acetate–hexanes). The product was further purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 40→90% acetonitrile in water, flow rate: 15 mL/min) to provide after concentration the pure α-glycoside **121** (4.6 mg, 30%).

| | | |
|---|---|---|
| **$^{1}$H NMR**: (500 MHz, $CDCl_3$) | 14.72 (s, 1H), 7.44 (s, 1H), 5.91 (br t, 1H, *J* = 2.4 Hz), 5.24 (d, 1H, *J* = 2.3 Hz), 5.22 (d, 1H, *J* = 4.1 Hz), 5.19 (d, 1H, *J* = 4.1 Hz), 4.75 (s, 1H), 4.41 (q, 1H, *J* = 6.4 Hz), 4.11 (ddd, 1H, *J* = 11.9, 5.5, 2.8 Hz), 3.78 (s, 3H), 3.63 (s, 3H), 3.47 (s, 3H), 3.45 (s, 1H), 3.04 (m, 2H), 2.89 (d, 1H, *J* = 6.0 Hz), 2.82 (d, 1H, *J* = 6.0 Hz), 2.73 (s, 2H), 2.58 (s, 3H), 2.18 (s, 3H), 2.16 (m, 1H), 2.09 (m, 2H), 1.15 (d, 1H, *J* = 6.9 Hz). | |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 204.4, 170.5, 162.9, 151.6, 142.4, 141.8, 135.2, 130.4, 115.8, 113.2, 113.1, 111.1, 104.5, 101.6, 99.6, 93.3, 77.2, 71.9, 70.1, 69.2, 68.3, 66.4, 60.9, 56.9, 56.4, 54.2, 47.7, 38.8, 23.6, 22.1, 20.8, 20.3, 16.8. | |
| **FTIR**, $cm^{-1}$: (neat) | 2926 (m), 2102 (m), 1748 (m), 1662 (m), 1389 (m), 1233 (m), 1016 (s). | |
| **HRMS**: (ESI) | Calcd for $(C_{33}H_{37}O_{13}N_3+H)^+$ | 684.2399 |
| | Found | 684.2424 |
| **TLC**: (80% ethyl acetate–hexanes) | $R_f$ = 0.77 (UV, CAM) | |




Azido Glycoside **126**.

2,3-dichloro-5,6-dicyanobenzoquinone (11 mg, 47 μmol, 1.0 equiv) was added to a solution of trimethylsilyl hemiketal **122** (39 mg, 47 μmol, 1.0 equiv) in a mixture of dichloromethane (1.7 mL) and water (330 μL) at 23 °C. The flask was covered with aluminum foil to exclude light. Over the course of 2 h, the color of the reaction mixture changed from myrtle green to yellow. The product solution was partitioned between water (10 mL) and dichloromethane (50 mL). The layers were separated. The aqueous layer was extracted with dichloromethane (3 × 20 mL). The organic layers were combined and the combined solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 40→90% acetonitrile in water, flow rate: 15 mL/min) to provide after concentration the pure benzylic alcohol **123** (30 mg, 90%).

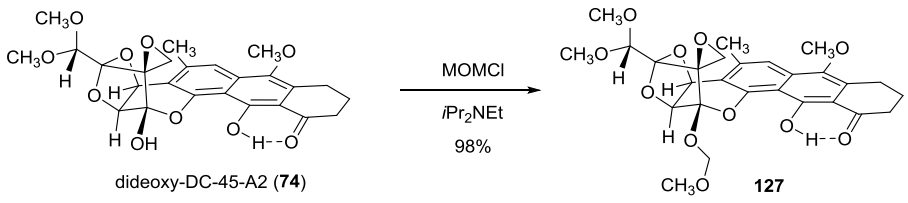
Thioglycoside **124**[77] (16 mg, 0.051 mmol, 6.0 equiv) was added to a solution of benzylic alcohol **123** (6 mg, 0.009 mmol, 1 equiv) in benzene (1.0 mL). The solution was concentrated and the residue was blanketed with argon. Dichloromethane (250 μL), crushed 4-Å molecular sieves (50 mg), and 2,6-di-*tert*-butyl-4-methylpyridine (7.0 mg, 0.034 mmol, 4 equiv) were added sequentially at 23 °C. After 10 min, the suspension was cooled to 0 °C. Silver hexafluorophosphate (4.3 mg, 0.017 mmol, 2.0 equiv) was added. After 15 min, an additional portion of silver hexafluorophosphate (4.3 mg, 0.017 mmol, 2.0 equiv) was added. After 12 h, additional portions of silver hexafluorophosphate (4.3 mg, 0.017 mmol, 2.0 equiv) and 2,6-di-*tert*-butyl-4-methylpyridine (3.5 mg, 0.017 mmol, 2 equiv) were added. After 20 min, dichloromethane (5 mL) was added and the mixture was filtered through short pad of Celite. The filtrate was partitioned between dichloromethane (30 mL) and water (5 mL). The layers were separated. The organic layer was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 40→100% acetonitrile in water, flow rate: 15 mL/min) to provide after concentration the pure α-glycoside **125** (2.3 mg, 30%).

Triethylamine–trihydrofluoride (13 μL, 76 μmol, 30 equiv) was added to an ice-cooled solution of α-glycoside **125** (2.3 mg, 2.6 μmol, 1 equiv) in acetonitrile (0.5 mL). The flask was covered with aluminum foil to exclude light. After 5 min, the cooling bath was removed and the reaction flask was allowed to warm to 23 °C. After 23 h, the

[77] Thioglycoside **124** was prepared from daunosamine hydrochloride by a three-step sequence which has been described previously: Zhang, G.; Fang, L.; Zhu, L.; Aimiuwu , J. E.; Shen, J.; Cheng, H.; Muller , M. T.; Lee, G. E.; Sun, D.; Wang, P. G. *J. Med. Chem.* **2005**, *48*, 5269–5278.

solution was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 20→90% acetonitrile in water, flow rate: 15 mL/min) to provide after concentration the pure alcohol **126** (0.55 mg, 30%).

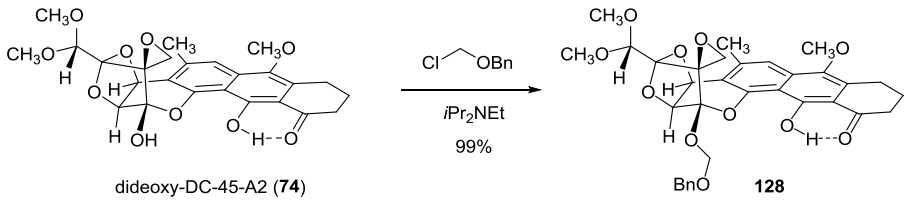
| | | |
|---|---|---|
| **$^1$H NMR**: (600 MHz, $CDCl_3$) | 14.20 (s, 1H), 7.50 (s, 1H), 5.43 (d, 1H, *J* = 2.8 Hz), 5.35 (t, 1H, *J* = 2.8 Hz), 5.27 (d, 1H, *J* = 4.1 Hz), 5.19 (s, 1H), 4.86 (dd, 1H, *J* = 12.8, 5.5 Hz), 4.84 (d, 1H, *J* = 4.1 Hz), 4.71 (s, 1H), 4.39 (s, 1H), 4.17 (q, 1H, *J* = 6.4 Hz), 3.84 (s, 3H), 3.79 (ddd, 1H, *J* = 12.8, 4.6, 2.8 Hz), 3.62 (s, 3H), 3.59 (s, 1H), 3.47 (s, 3H), 3.16 (d, 1H, *J* = 5.5 Hz), 3.03 (d, 1H, *J* = 5.0 Hz), 2.72 (ddd, 1H, *J* = 13.3, 5.0, 3.2 Hz), 2.62 (s, 3H), 2.23–2.17 (m, 1H), 2.19 (s, 3H), 2.08 (dt, 1H, *J* = 12.8, 3.6 Hz), 1.78 (dd, 1H, *J* = 12.8, 4.6 Hz), 1.25 (d, 3H, *J* = 6.4 Hz). | |
| **HRMS**: (ESI) | Calcd for $(C_{33}H_{37}O_{15}N_3+Na)^+$ | 738.2117 |
| | Found | 738.2084 |




Methoxymethyl Aglycon **127**.

Chloromethyl methyl ether (26 μL, 0.339 mmol, 5.0 equiv) was added to a solution of dideoxy-DC-45-A2 (**74**) (33 mg, 0.068 mmol, 1 equiv) and *N*,*N*-diisopropylethylamine (118 μL, 0.678 mmol, 10 equiv) in dichloromethane (1.4 mL) at 23° C. After 25 min, the mixture was partitioned between pH 7 aqueous phosphate buffer (10 mL) and dichloromethane (30 mL). The layers were separated. The organic layer was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide methoxymethyl aglycon **127** (36 mg, >99%).

| | | |
|---|---|---|
| **$^1$H NMR**: (500 MHz, $CDCl_3$) | 14.76 (s, 1H), 7.44 (s, 1H), 5.53 (d, 1H, *J* = 6.6 Hz), 5.30 (s, 1H), 5.20 (d, 1H, *J* = 4.0 Hz), 5.14 (d, 1H, *J* = 4.6 Hz), 4.93 (d, 1H, *J* = 7.2 Hz), 4.77 (s, 1H), 3.77 (s, 3H), 3.64 (s, 3H), 3.57 (s, 3H), 3.46 (s, 3H), 3.03 (m, 2H), 2.91 (d, 1H, *J* = 5.3 Hz), 2.85 (d, 1H, *J* = 5.9 Hz), 2.72 (m, 2H), 2.59 (s, 3H), 2.09 (m, 2H). | |
| **HRMS**: (ESI) | Calcd for $(C_{27}H_{30}O_{11}+H)^+$ | 531.1861 |
| | Found | 531.1872 |

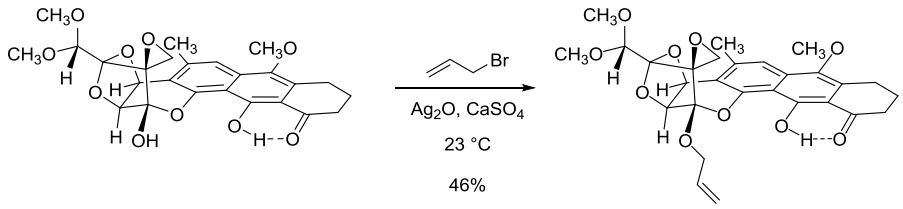



Benzyloxymethyl Aglycon **128**.

Benzyl chloromethyl ether (4.3 μL, 0.031 mmol, 5 equiv) was added to a solution of dideoxy-DC-45-A2 (**74**) (3.0 mg, 6.2 μmol, 1 equiv) and *N*,*N*-diisopropylethylamine (10.8 μL, 0.062 mmol, 10 equiv) in dichloromethane (0.1 mL) at 23 °C. After 30 min, the mixture was partitioned between half-saturated aqueous sodium chloride solution (5 mL) and dichloromethane (15 mL). The layers were separated and the organic layer was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was purified by flash-column chromatography (20% ethyl acetate–hexanes initially, grading to 50% ethyl acetate–hexanes) to provide benzyloxymethyl aglycon **128** (3.7 mg, 99%).

**$^1$H NMR**: (500 MHz, $CDCl_3$) 14.76 (s, 1H), 7.43 (s, 1H), 7.38–7.26 (m, 5H), 5.64 (d, 1H, $J$ = 7.0 Hz), 5.21 (d, 1H, $J$ = 4.0 Hz), 5.18 (d, 1H, $J$ = 4.0 Hz), 5.11 (d, 1H, $J$ = 7.3 Hz), 4.99 (d, 1H, $J$ = 11.7 Hz), 4.79 (s, 1H), 4.72 (d, 1H, $J$ = 11.7 Hz), 3.77 (s, 3H), 3.66 (s, 3H), 3.47 (s, 3H), 3.04 (m, 2H), 2.93 (d, 1H, $J$ = 5.5 Hz), 2.87 (d, 1H, $J$ = 5.5 Hz), 2.72 (m, 2H), 2.58 (s, 3H), 2.09 (m, 2H).

| | | |
|---|---|---|
| **HRMS**: | Calcd for $(C_{33}H_{35}O_{11}+K)^+$ | 645.1733 |
| (ESI) | Found | 645.1725 |



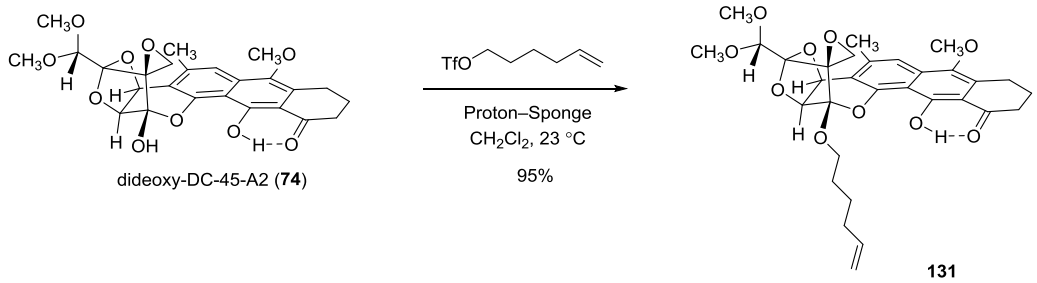

dideoxy-DC-45-A2 (**74**) **130**

Allyl Aglycon **130**.[75]

Silver(I) oxide (29 mg, 0.12 mmol, 2.0 equiv) was added to a suspension of dideoxy-DC-45-A2 (**74**), allyl bromide (2 mL), and calcium sulfate (34 mg, 0.25 mmol, 4.0 equiv) at 23 °C. After 21 h, the mixture was filtered through a short pad of Celite, eluting with ethyl acetate. The filtrate was concentrated. The residue was purified by preparatory HPLC (Agilent Prep-C18 column, 10 μm, 30 × 150 mm, UV detection at 270 nm, gradient elution with 40→90% acetonitrile in water, flow rate: 15 mL/min) to provide after concentration the pure allyl aglycon **130** (14.9 mg, 46%).

| | |
|---|---|
| **$^{1}$H NMR**: (500 MHz, $CDCl_3$) | 14.79 (s, 1H), 7.43 (s, 1H), 6.10 (m, 1H), 5.39 (d, 1H, $J$ = 17.5 Hz), 5.23–5.20 (m, 2H), 4.81 (d, 1H, $J$ = 4.4 Hz), 4.76 (s, 1H), 4.54 (m, 2H), 3.78 (s, 3H), 3.63 (s, 3H), 3.44 (s, 3H), 3.04 (m, 2H), 2.89 (d, 1H, $J$ = 5.6 Hz), 2.86 (d, 1H, $J$ = 6.0 Hz), 2.73 (m, 2H), 2.59 (s, 3H), 2.09 (m, 2H). |
| **$^{13}$C NMR**: (125 MHz, $CDCl_3$) | 204.4, 163.0, 151.3, 142.4, 141.8, 135.2, 134.6, 130.2, 117.4, 115.6, 113.5, 111.0, 110.7, 104.4, 102.0, 99.8, 72.6, 69.3, 68.9, 66.7, 60.9, 57.0, 56.7, 48.2, 38.8, 23.6, |

22.1, 20.4.




5-Hexenyl Aglycon **131**.

4-Å molecular sieves (~20 mg) were added to a solution of dideoxy-DC-45-A2 (**74**) (25 mg, 0.051 mmol, 1 equiv) and 1,8-bis(dimethylamino)naphthalene ("Proton–Sponge", 165 mg, 0.771 mmol, 15 equiv) in dichloromethane (0.6 mL) at 23 °C. After 20 min, 5-hexenyl trifluoromethanesulfonate (120 μL) was added. After 20 h, the mixture was partitioned between saturated aqueous sodium bicarbonate solution (20 mL) and dichloromethane (50 mL). The layers were separated and the organic layer was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue was immediately purified by flash-column chromatography (10% ethyl acetate–hexanes initially, grading to 50% ethyl acetate–hexanes). The fractions containing product were concentrated. The residue was dissolved in ethyl acetate (30 mL), and the resulting solution was washed successively with 0.1 M aqueous hydrochloric acid solution (2 × 20 mL), water (20 mL), and saturated aqueous sodium chloride solution (20 mL). The washed organic solution was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the pure 5-hexenyl aglycon **131** (25 mg, 86%).

**$^1$H NMR**: 14.78 (s, 1H), 7.42 (s, 1H), 5.84 (m, 1H), 5.21 (d, 1H, *J* =

| | | |
|---|---|---|
| (500 MHz, $CDCl_3$) | 3.8 Hz), 5.03 (d, 1H, *J* = 17.6 Hz), 4.95 (d, 1H, *J* = 9.9 Hz), 4.78 (d, 1H, *J* = 3.8 Hz), 4.75 (s, 1H), 3.98 (m, 2H), 3.78 (s, 3H), 3.63 (s, 3H), 3.44 (s, 3H), 3.04 (m, 2H), 2.86 (m, 2H), 2.73 (m, 2H), 2.58 (s, 3H), 2.10 (m, 4H), 1.76 (m, 2H), 1.55 (m, 2H). | |
| **$^{13}$C NMR**: | 204.4, 163.0, 151.5, 142.3, 141.8, 138.9, 135.2, 130.1, | |
| (125 MHz, $CDCl_3$) | 115.5, 114.4, 113.4, 113.2, 110.9, 104.4, 102.0, 99.8, 72.1, 69.2, 68.9, 65.3, 60.8, 56.9, 56.7, 48.1, 38.8, 33.5, 29.4, 25.3, 23.5, 22.1, 20.4. | |
| **FTIR**, $cm^{-1}$: | 2942 (m), 1620 (s), 1389 (s), 1092 (s). | |
| (neat) | | |
| **HRMS**: | Calcd for $(C_{31}H_{36}O_{10}+H)^+$ | 569.2381 |
| (ESI) | Found | 569.2375 |
| **TLC**: | $R_f$ = 0.84 (UV, CAM) | |
| (5% methanol–dichloromethane) | | |

# Chapter 5

# Antiproliferative Activities of Fully Synthetic Trioxacarcins

## Introduction

An additional goal of our pursuit of diverse trioxacarcin analogs was to begin to define the necessary structural features for potent antiproliferative activity. Accordingly, I measured the antiproliferative activities of many of the fully synthetic analogs discussed in this dissertation. In this chapter, I provide these measurements of antiproliferative activity, reported as the $GI_{50}$,[78] the concentration necessary for 50% growth inhibition. The cell proliferation assay was performed using cultured H460 cells, a human lung cancer cell line that was reported to be especially sensitive to trioxacarcin A (**1**)[4] (which I confirmed, obtaining a $GI_{50}$ value of 0.85 ± 0.36 nM). I also made the first measurement, to our knowledge, of the growth-inhibitory activity of the natural product DC-45-A1 (**10**) ($GI_{50}$ 37 ± 7 nM). After tabulating these measurements, I highlight trends relevant to the structure-activity relationship which our program in analog synthesis provided a unique ability to observe.

## Antiproliferative Activities

Table 5.1 lists the antiproliferative activities measured for fully synthetic trioxacarcins discussed in this dissertation, ordered by their structure number. Table 5.2 lists the same antiproliferative activities sorted in order of increasing $GI_{50}$.

[78] Boyd, M. R.; Pauli, K. D. *Drug Develop. Res.* **1995**, *34*, 91–109.

**Table 5.1.** Antiproliferative Activities ($GI_{50}$) of Fully Synthetic Trioxacarcins, Sorted by Structure Number.

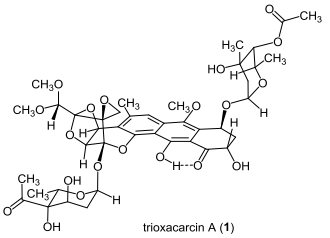
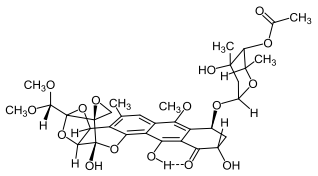
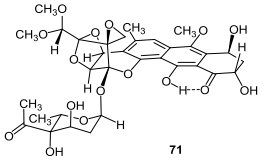
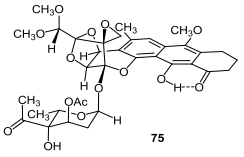
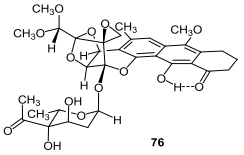
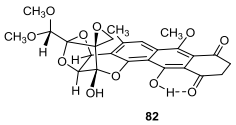
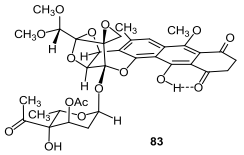
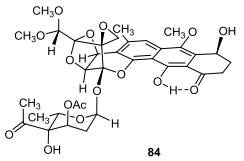
| Structure | $GI_{50}$ (H460 cells, nM) |
|---|---|
|  trioxacarcin A (**1**) | 0.85 ± 0.36 |
|  DC-45-A1 (**10**) | 37 ± 7 |
|  **71** | 767 ± 74 |
|  **75** | 4.1 ± 1.7 |
|  **76** | 19 ± 7 |
|  **82** | 105 ± 24 |
|  **83** | 18 ± 8 |
|  **84** | 294 ± 52 |

Table 5.1, Continued

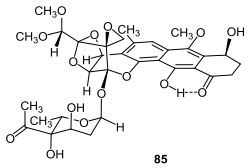
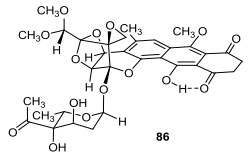
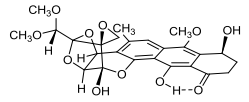
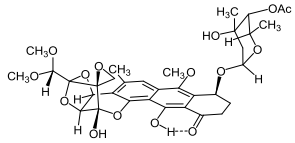
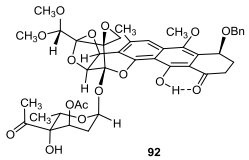
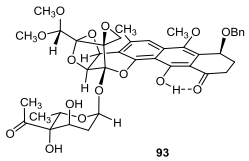
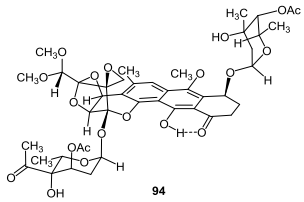
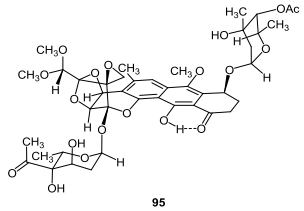
| Structure | $GI_{50}$ (H460 cells, nM) |
|---|---|
|  **85** | 206 ± 70 |
|  **86** | 10.1 ± 0.4 |
|  **89** | 200 ± 57 |
|  **90** | 17 ± 2 |
|  **92** | 679 ± 118 |
|  **93** | 315 ± 58 |
|  **94** | 56 ± 19 |
|  **95** | 4.5 ± 2.0 |

Table 5.1, Continued

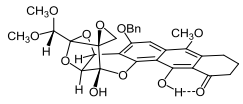
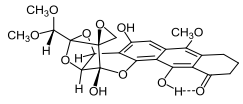
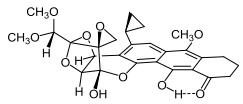
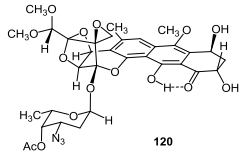
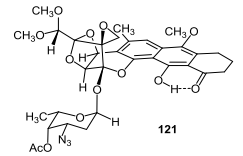
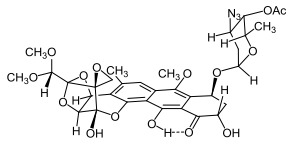
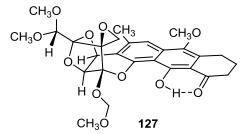
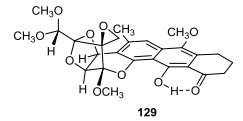
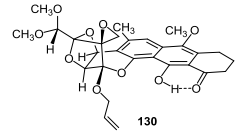
| Structure | $GI_{50}$ (H460 cells, nM) |
|---|---|
|  101 | >2500 |
|  102 | >2500 |
|  112 | 374 ± 87 |
|  120 | 397 ± 82 |
|  121 | 102 ± 3 |
|  126 | 225 ± 26 |
|  127 | 2.4 ± 0.9 |
|  129 | 2.6 ± 0.4 |
|  130 | < 1.1 |

**Table 5.2.** Antiproliferative Activities ($GI_{50}$) of Fully Synthetic Trioxacarcins, Sorted in Increasing Order of $GI_{50}$.

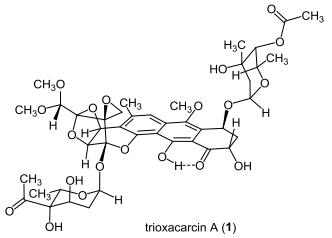
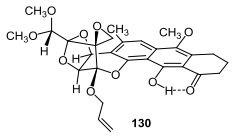
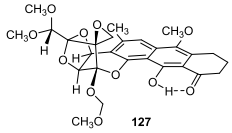
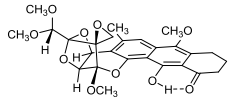
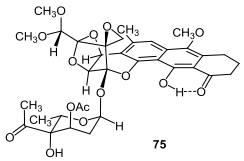
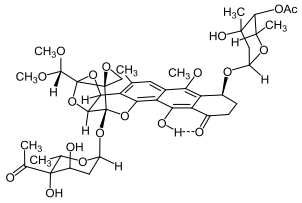
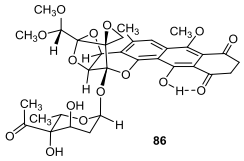
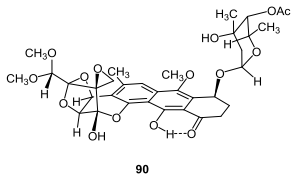
| Structure | $GI_{50}$ (H460 cells, nM) |
|---|---|
|  trioxacarcin A (**1**) | 0.85 ± 0.36 |
|  **130** | < 1.1 |
|  **127** | 2.4 ± 0.9 |
|  **129** | 2.6 ± 0.4 |
|  **75** | 4.1 ± 1.7 |
|  **95** | 4.5 ± 2.0 |
|  **86** | 10.1 ± 0.4 |
|  **90** | 17 ± 2 |

Table 5.2, Continued

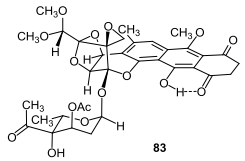
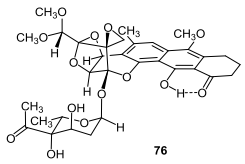
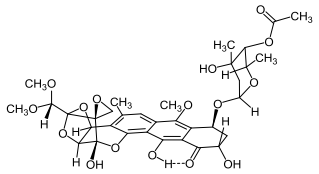
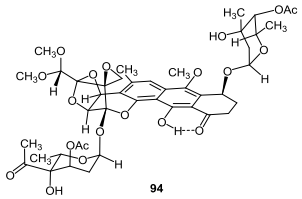
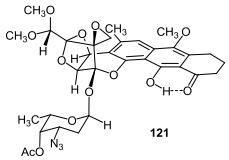
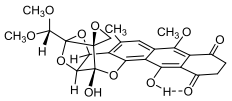
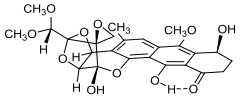
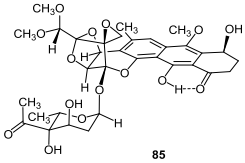
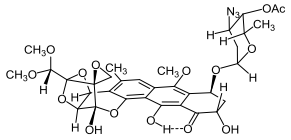
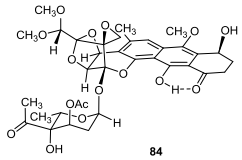
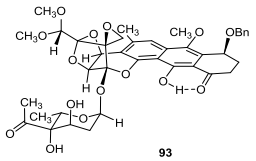
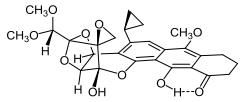
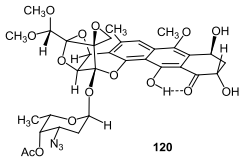
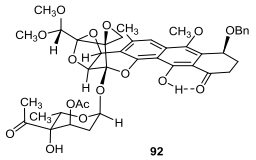
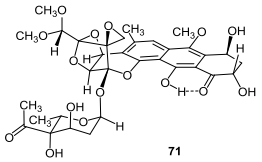
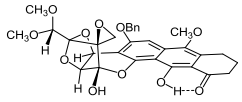
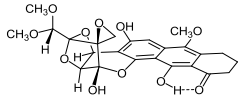
| Structure | $GI_{50}$ (H460 cells, nM) |
|---|---|
|  **83** | 18 ± 8 |
|  **76** | 19 ± 7 |
|  DC-45-A1 (**10**) | 37 ± 7 |
|  **94** | 56 ± 19 |
|  **121** | 102 ± 3 |
|  **82** | 105 ± 24 |
|  **89** | 200 ± 57 |
|  **85** | 206 ± 70 |

Table 5.2, Continued

| Structure | $GI_{50}$ (H460 cells, nM) |
|---|---|
|  **126** | 225 ± 26 |
|  **84** | 294 ± 52 |
|  **93** | 315 ± 58 |
|  **112** | 374 ± 87 |
|  **120** | 397 ± 82 |
|  **92** | 679 ± 118 |
|  **71** | 767 ± 74 |
|  **101** | >2500 |
|  **102** | >2500 |

## Discussion

Our synthetic efforts provided us with access not only to trioxacarcin A (**1**) but also to its 4-deglyco analog DC-45-A1 (**10**) and its previously unknown 13-deglyco analog, monoglycoside **71**. Both DC-45-A1 (**10**) and **71** were substantially less active in our antiproliferative assay than trioxacarcin A (Figure 5.1). Still, DC-45-A1 (**10**), which contains only the trioxacarcinose A sugar, was ~20 times more potent than monoglycoside **71**, which contains only the trioxacarcinose B sugar. Both of these were also far more potent than the trioxacarcin aglycon, DC-45-A2 (**11**).[18] The presence of the trioxacarcinose A and B sugar residues clearly imparts high potency relative to the presence of the corresponding free hydroxyl and hemiketal groups, at least in this assay.

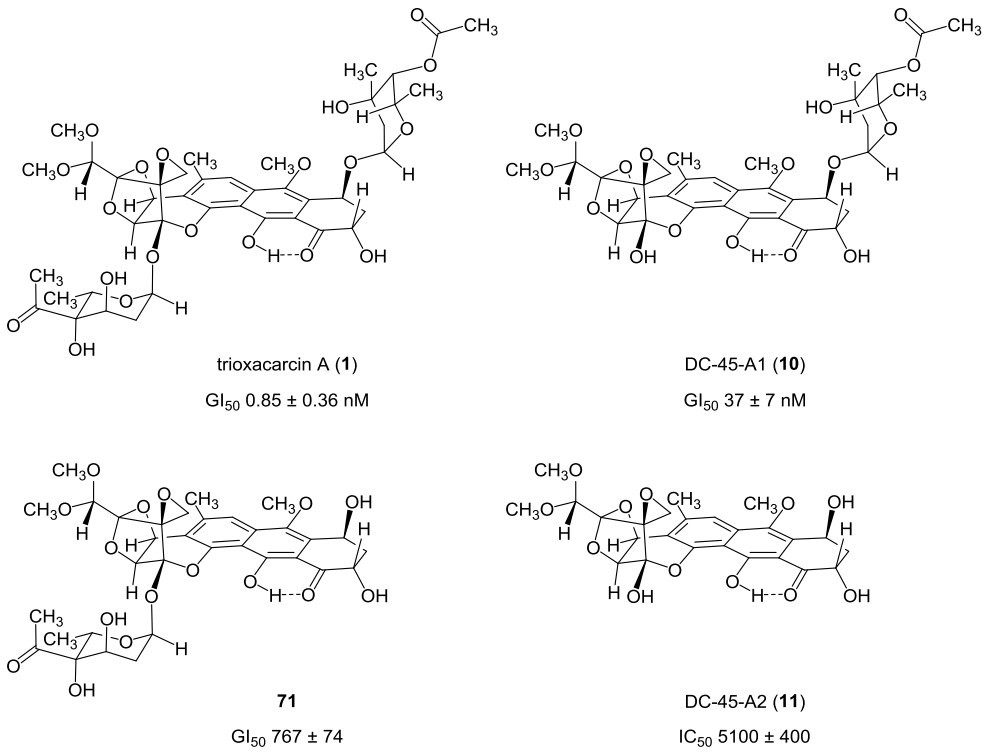



**Figure 5.1.** Comparison of $GI_{50}$ Values for the Natural Products Trioxacarcin A (**1**), DC-45-A1 (**10**), and DC-45-A2 (**11**),[18] Alongside Analog **71**.

Another finding that can be extracted from our measurements is the striking effect on antiproliferative activity of changes in the substitution of the cyclohexenone ring within the dihydroanthracenone chromophore. Figure 5.2 compares the $GI_{50}$ values of four compounds differing only in their C2 and C4 substituents. Relative to monoglycoside **71**, 2-deoxy monoglycoside **85** lacks a C2 hydroxyl group and was more active in our assay; the 2,4-dideoxy analog **76** and the diketone **86** were even more potent. This mirrors the earlier observation that dideoxy-DC-45-A2 (**74**) is a much more potent antiproliferative agent than DC-45-A2 (**11**).[18]

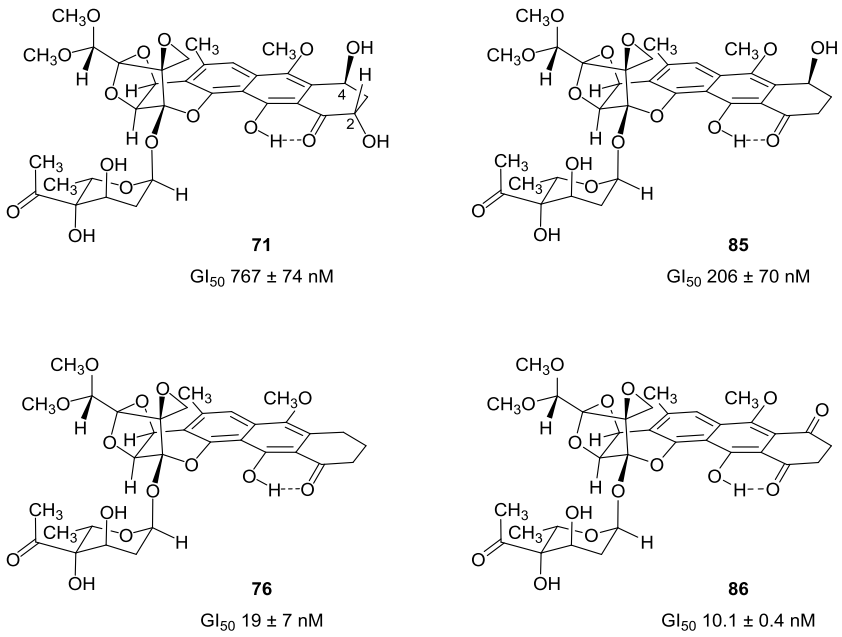



**Figure 5.2.** Antiproliferative Activities of Analogs **71**, **85**, **76**, and **86**, which Differ in Cyclohexenone Ring Substitution.

The analog 2-deoxy-DC-45-A1 (**90**) was, interestingly, roughly 2 times more active than the natural product DC-45-A1 (**10**) itself. 2-Deoxytrioxacarcin A (**95**), while still an exceptionally potent antiproliferative agent, was less active than trioxacarcin A

(**1**), an exception to the broad trend of increasing activity through the removal of C2 or C4 hydroxyl groups.

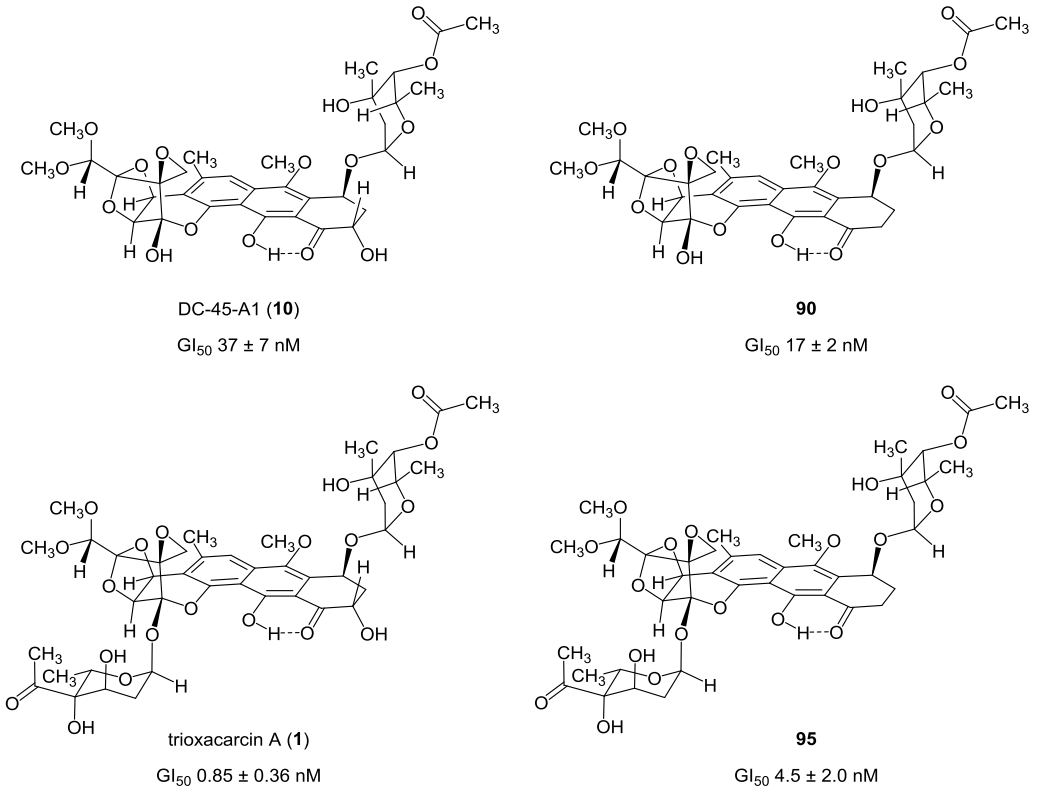



**Figure 5.3.** Comparison of the Antiproliferative Activities of DC-45-A1 (**10**) and Trioxacarcin A (**1**) with the Corresponding 2-Deoxy Analogs.

None of the small group of analogs prepared by modification of the cyanophthalide component (**19**)—namely, benzyl ether **101**, unstable phenol **102**, and cyclopropyl analog **112**—exhibited increased potency relative to dideoxy-DC-45-A2 (**74**),[18] which possesses the C6 methyl substituent normally present within the trioxacarcins (and is otherwise identical to **101**, **102**, and **112**). Benzyl ether **101** and phenol **102** showed no antiproliferative effects within the range of concentrations examined (Figure 5.4).

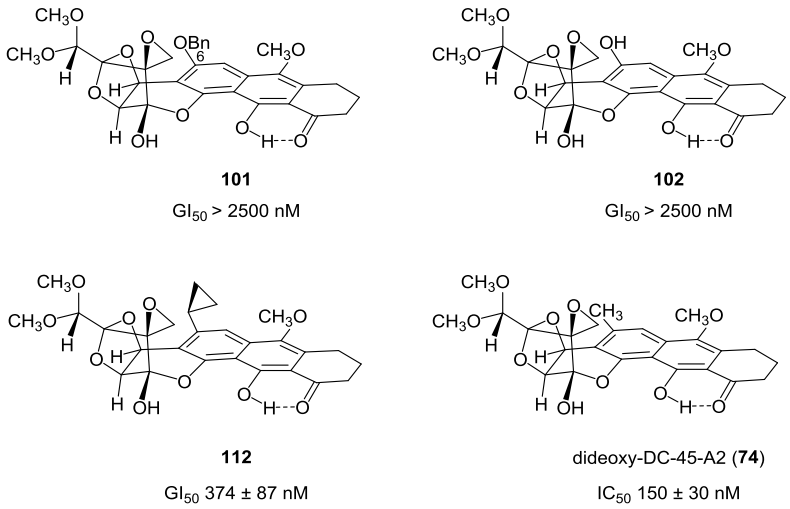



**Figure 5.4.** Antiproliferative Activities of Analogs Prepared By Variation of the Cyanophthalide Component (**19**), Alongside Dideoxy-DC-45-A2 (**74**).[18]

The azido sugar-containing analogs **120**, **121**, and **126** retained some antiproliferative activity (Figure 5.5). Interestingly, compound **120** was a modestly more active antiproliferative agent than the analogous structure bearing the natural trioxacarcinose B residue (**71**). This was not a general result of incorporation of this azido sugar, as analogs **121** and **126** are less active than their counterparts bearing the naturally occurring trioxacarcinoses.

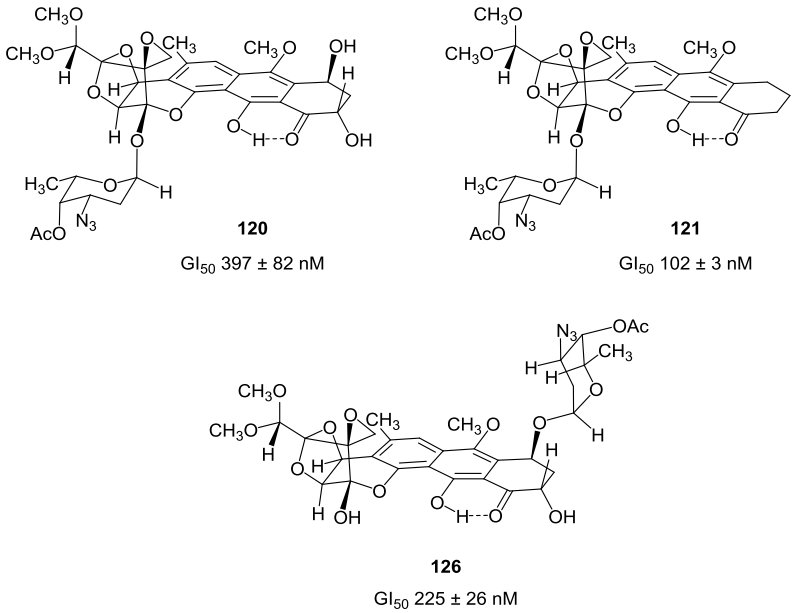



**Figure 5.5.** Antiproliferative Activities of Azido Sugar-Containing Analogs **120**, **121**, and **126**.

The most surprising results came in the form of the $GI_{50}$ values of C13 alkylated analogs **127**, **129**, and **130** (Figure 5.6). Each of these analogs was nearly two orders of magnitude more active than the corresponding free hemiketal, dideoxy-DC-45-A2 (**74**). These compounds represent our most potent trioxacarcin analogs to date and showed activities comparable to trioxacarcin A (**1**) despite their far simpler chemical structure. The reason for the remarkable activity of these compounds in particular is not immediately clear, though factors such as improved cellular membrane penetration, facilitated duplex DNA intercalation, improved kinetics of guanosine alkylation, and even improved chemical stability could all play a role, and further study will be required to determine the relative significance of these and other factors.

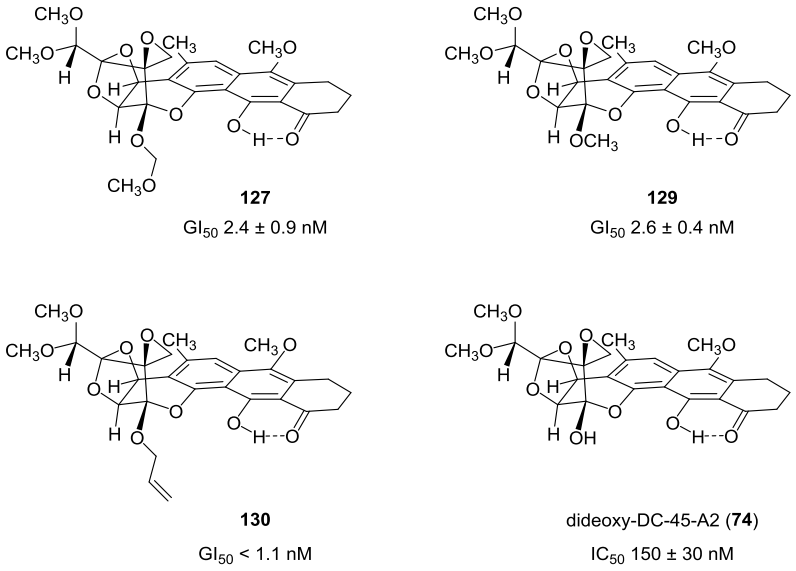



**Figure 5.6.** Antiproliferative Activities of C13-Alkylated Trioxacarcins, Compared to Dideoxy-DC-45-A2 (**74**).[18]

## Conclusion

Measurement of the antiproliferative activities of the novel trioxacarcins prepared using our synthetic route has begun to define the effects of structural variations around the polycyclic scaffold and within the glycosyl residues. The majority of the analogs we prepared were at least submicromolar inhibitors of the growth of a cultured human lung cancer cell line. We also identified several analogs which are considerably simpler than trioxacarcin A (**1**) yet retain highly potent antiproliferative activity.

In this dissertation, I have presented my work related to the development of a component-based synthetic route to trioxacarcins. I developed two efficient routes to the sugar trioxacarcinose A by diastereoselective allylmetal additions to ketones, and I used a

scalable dihydroxylation–recrystallization protocol to provide the common starting material for these two routes. An activated derivative of trioxacarcinose A was then used in a highly convergent synthesis of trioxacarcin A (**1**) from five components of similar synthetic complexity: epoxy diazo diketone **18**, cyanophthalide **19**, cyclohexenone **20**, trioxacarcinose A donor **55**, and trioxacarcinose B donor **65**. Subsequent efforts in trioxacarcin analog synthesis underscored the modularity of this route and produced a large and varied collection of novel trioxacarcins. These studies have significantly expanded the pool of trioxacarcin structures available for study. Finally, through measurements of antiproliferative activity we have begun to study the effects of particular structural modifications on the growth-inhibitory properties of trioxacarcins in cancer cells.

## Experimental Section

*Cell Culture.*

All cell-culture work was conducted in a class II biological safety cabinet. H460 cells (P2–P4, American Type Culture Collection) were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS).

*Cell Proliferation Assay*

H460 cells were grown to approximately 80% confluence, and then were trpysinized, collected, and pelleted by centrifugation (10 min at 183 × *g*, 4 °C). The supernatant was discarded and the cell pellet was resuspended in 10 mL of fresh medium. A sample was diluted 10-fold in fresh medium, and the concentration of cells was determined using a hemacytometer. The cell suspension was diluted to a concentration of 3000 cells/100 μL. The wells of a pre-sterilized 96-well plate were charged with 100 μL per well of the diluted cellular suspension. The plate was incubated for 24 h at 37 °C (5% $CO_2$).

Stock solutions of each compound in DMSO were diluted serially with RPMI-1640 medium (supplemented with FBS), and 100-μL aliquots of the resulting solutions were added to the wells containing adhered cells to achieve final concentrations of 0.11 nM to 250 nM (trioxacarcin A) or 1.1 nM to 2500 nM (all other compounds). After incubating at 37 ºC for 72 h (5% $CO_2$), 20 μL of resazurin solution (Promega CellTiter-Blue® Cell Viability Assay) was added to each well. After incubating at 37 °C for 2.0 h (5% $CO_2$), the fluorescence (544 nm excitation/590 nm emission) was recorded using a microplate reader (SpectraMax PLUS$^{384}$) as a measure of viable cells.

Percent growth inhibition was calculated for each well, based upon the following formula:

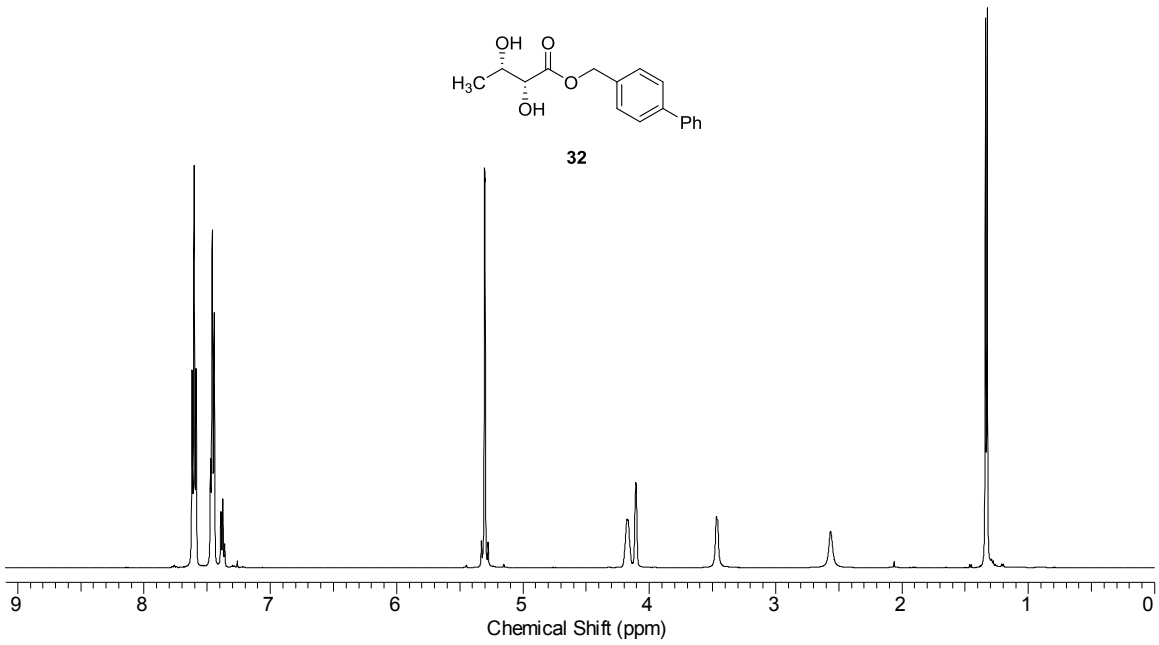
$$\text{Percent growth inhibition} = 100 \times (S - B_0) / (B_t - B_0)$$

where $S$ is the sample fluorescence, $B_t$ is the average fluorescence of an untreated population of cells at the completion of the assay, and $B_0$ is the average fluorescence of an untreated population of cells at the beginning of the assay.

Each compound was assayed at eight separate concentrations per experiment. The percent inhibition at each concentration was plotted against log(concentration), and a curve fit was generated using the XLfit4 plugin (IDBS Software) running in Excel (Microsoft). $GI_{50}$ values were computed to reflect the concentrations at which the resulting curves pass through 50% inhibition. $GI_{50}$ values for each compound are reported as the average of at least six experiments, with standard deviation.

# Appendix A

# Catalog of Spectra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3
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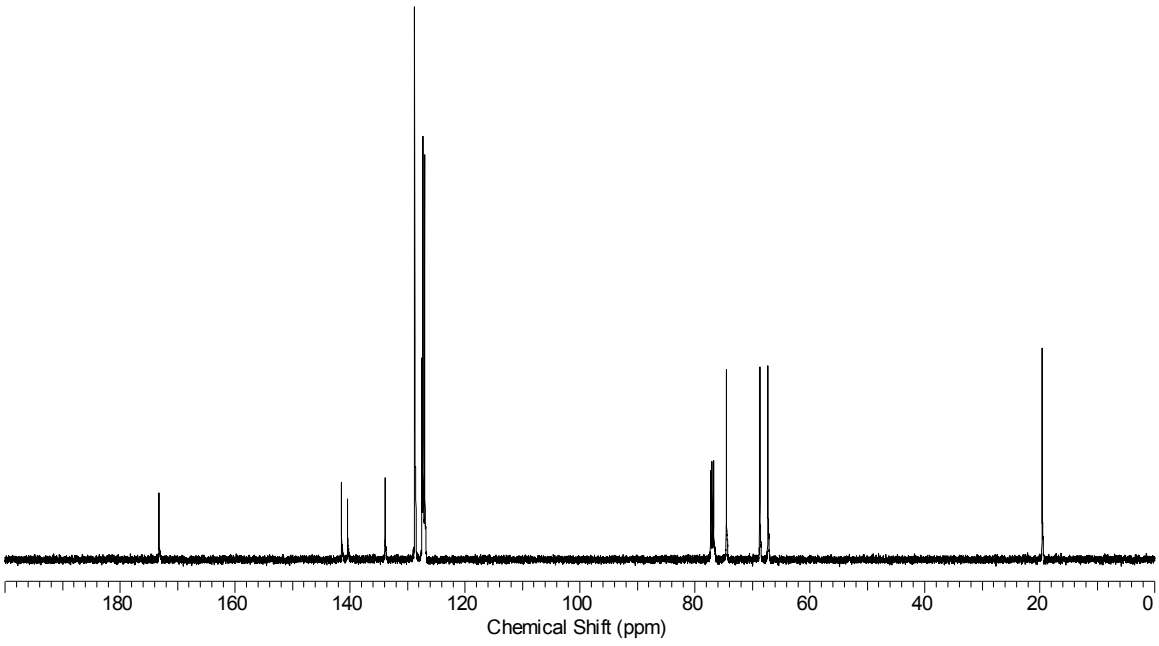
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100
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20
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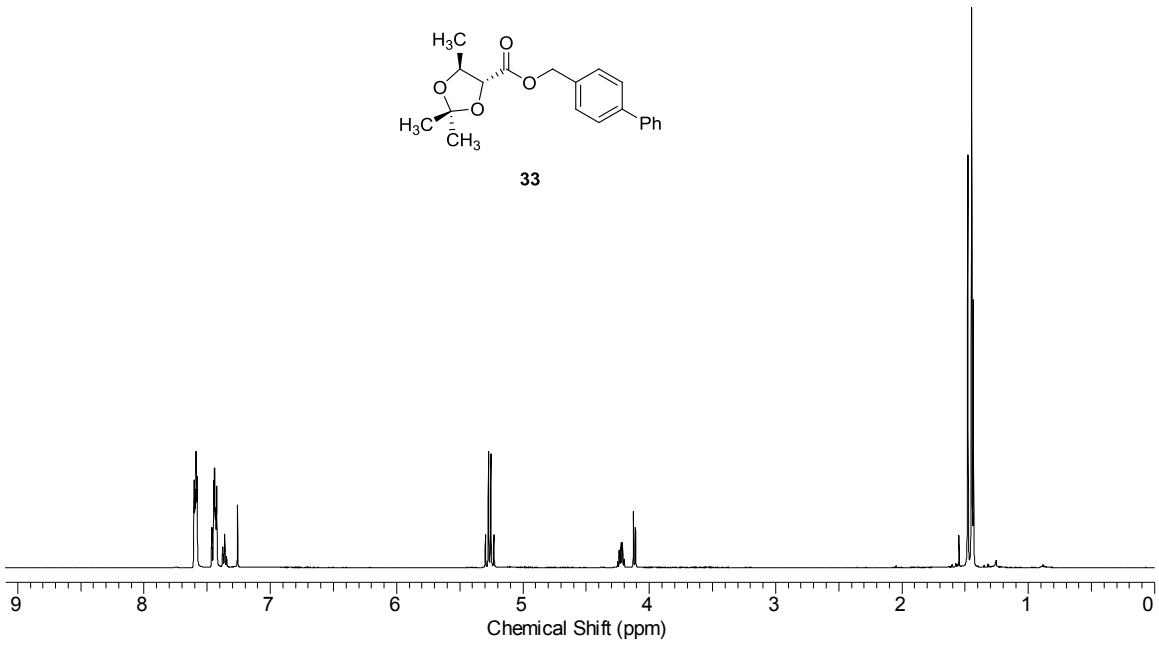
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O
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H3C
CH3
Ph
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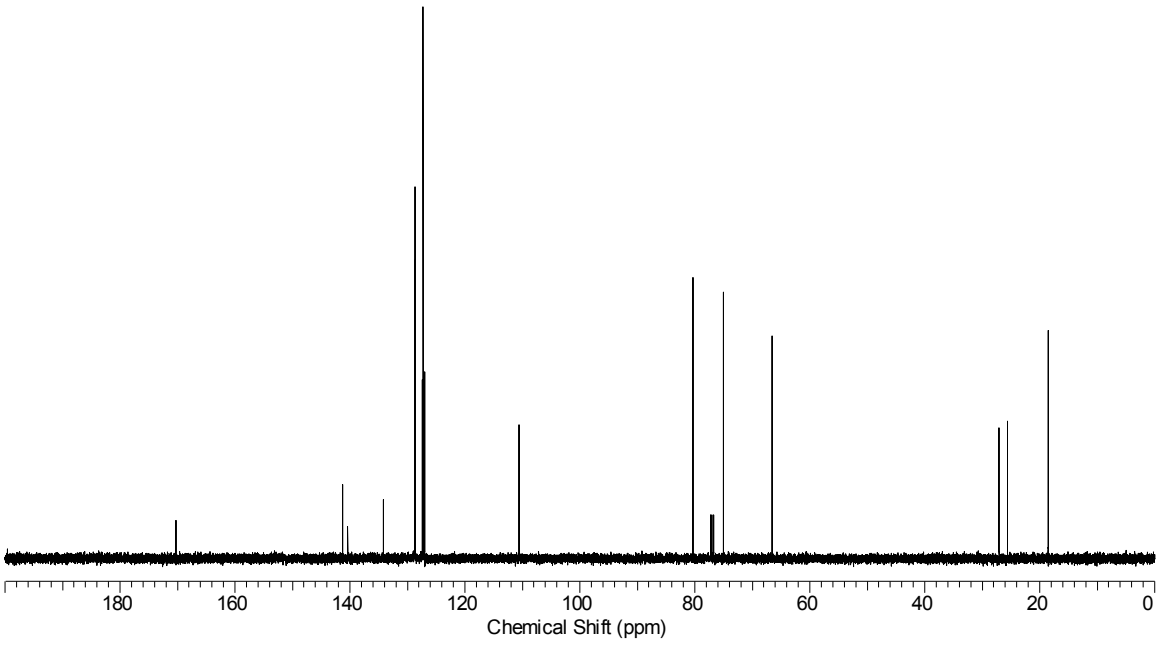
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

**33**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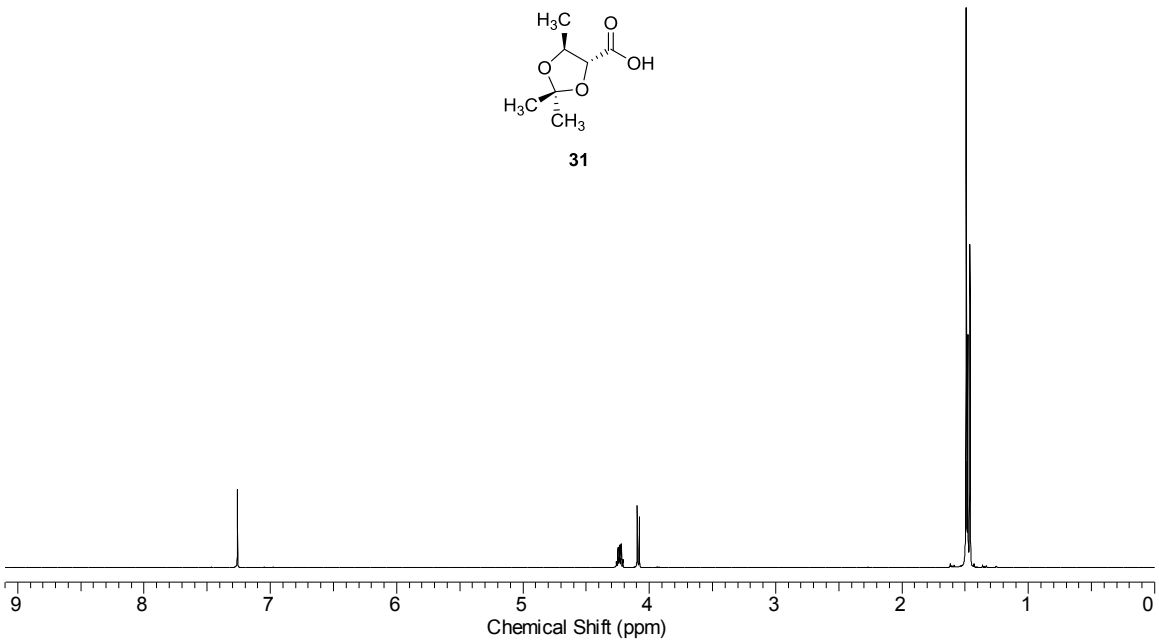
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H3C
CH3
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5
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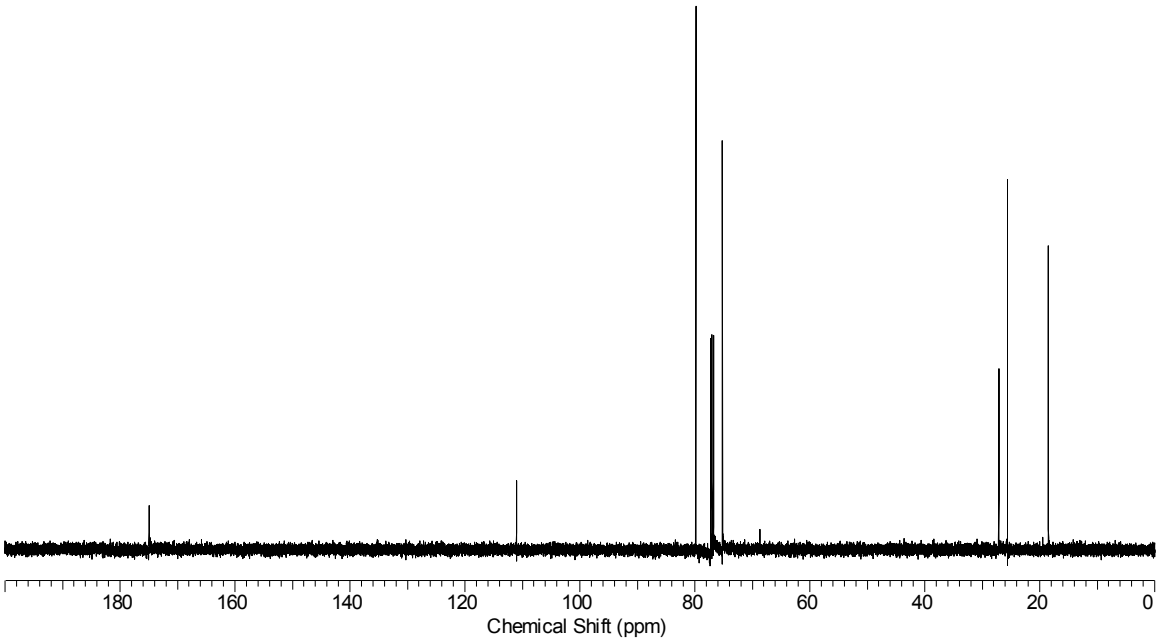
3
2
1
0
Chemical Shift (ppm)

**31**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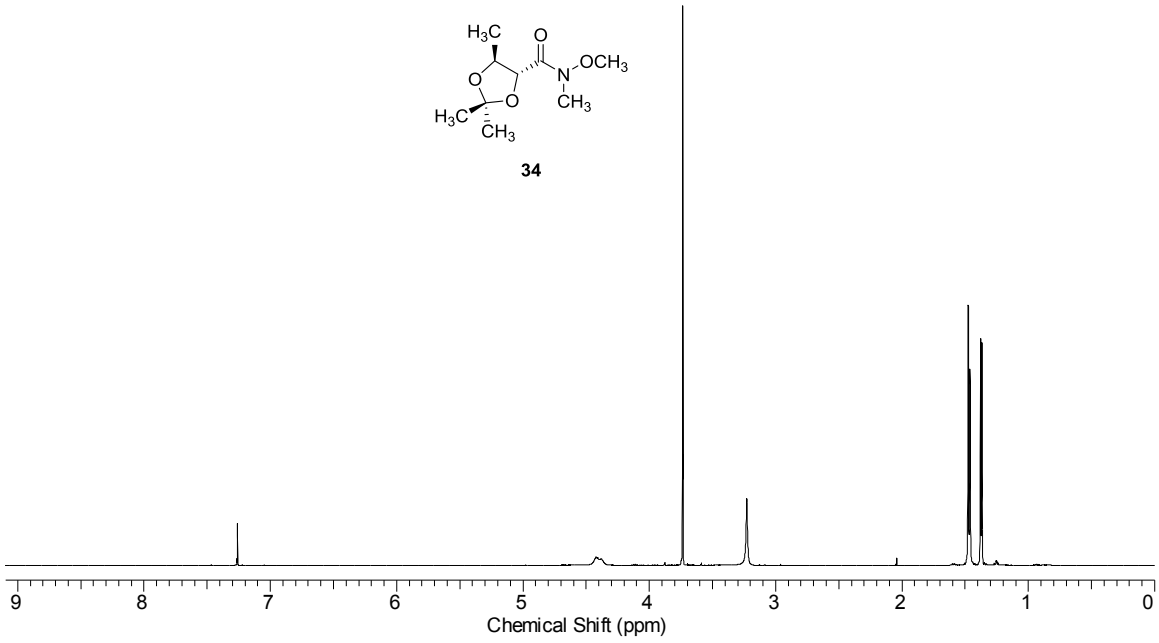
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CH3
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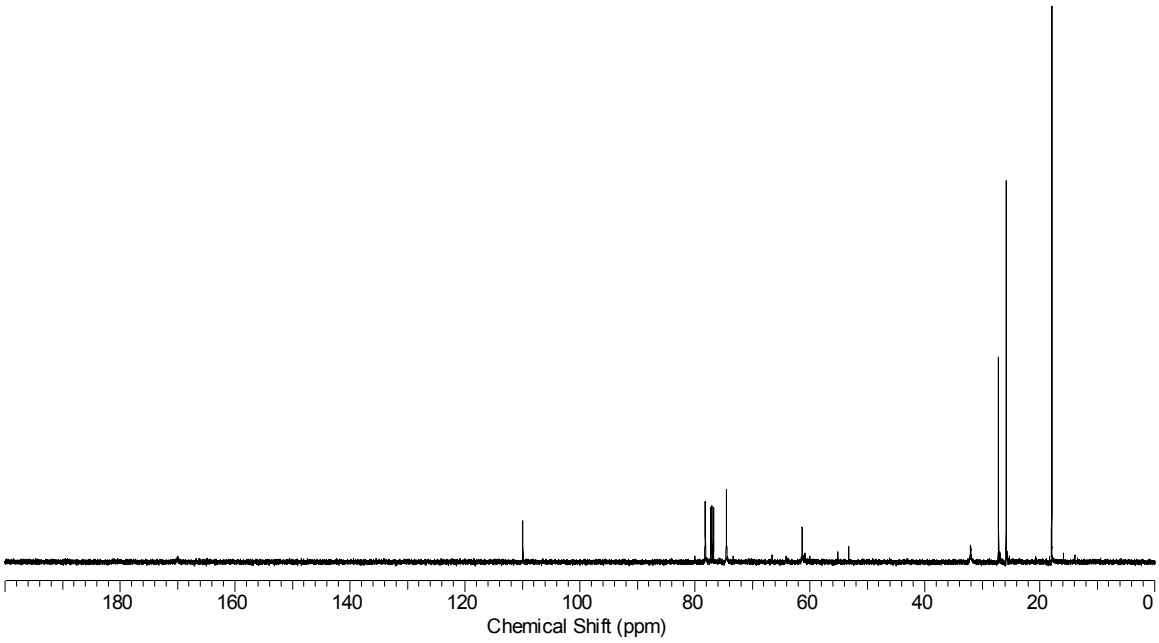
6
5
4
3
2
1
0
Chemical Shift (ppm)

34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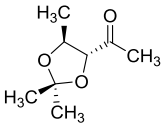
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O
CH3
O
O
H3C
CH3

**35**


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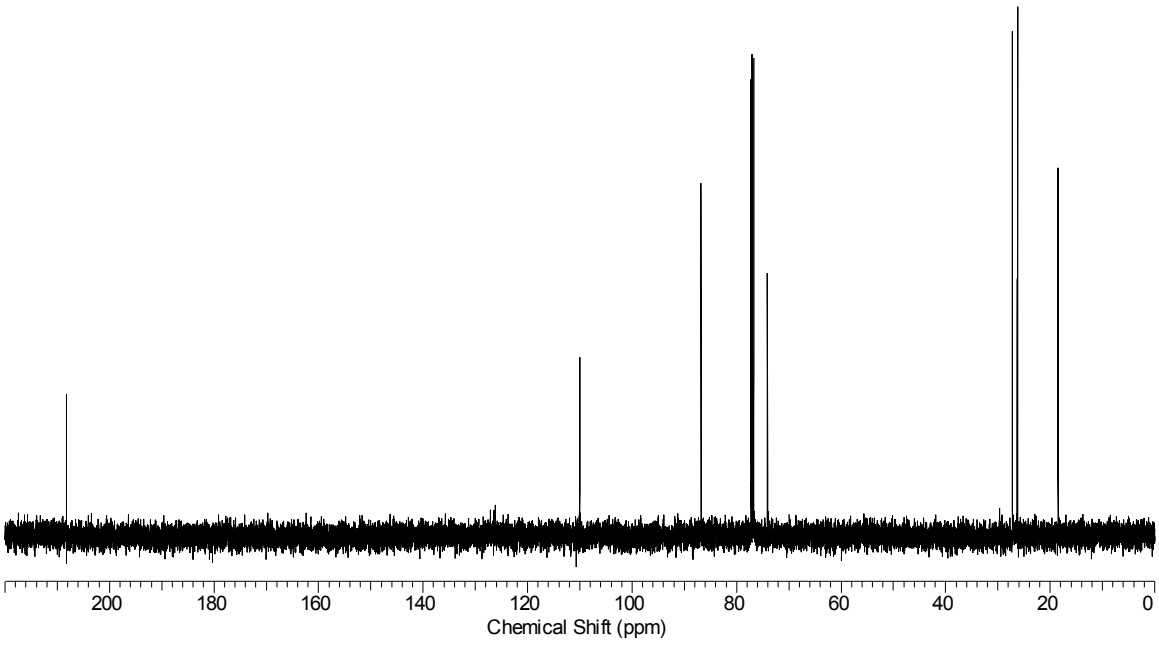
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)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100
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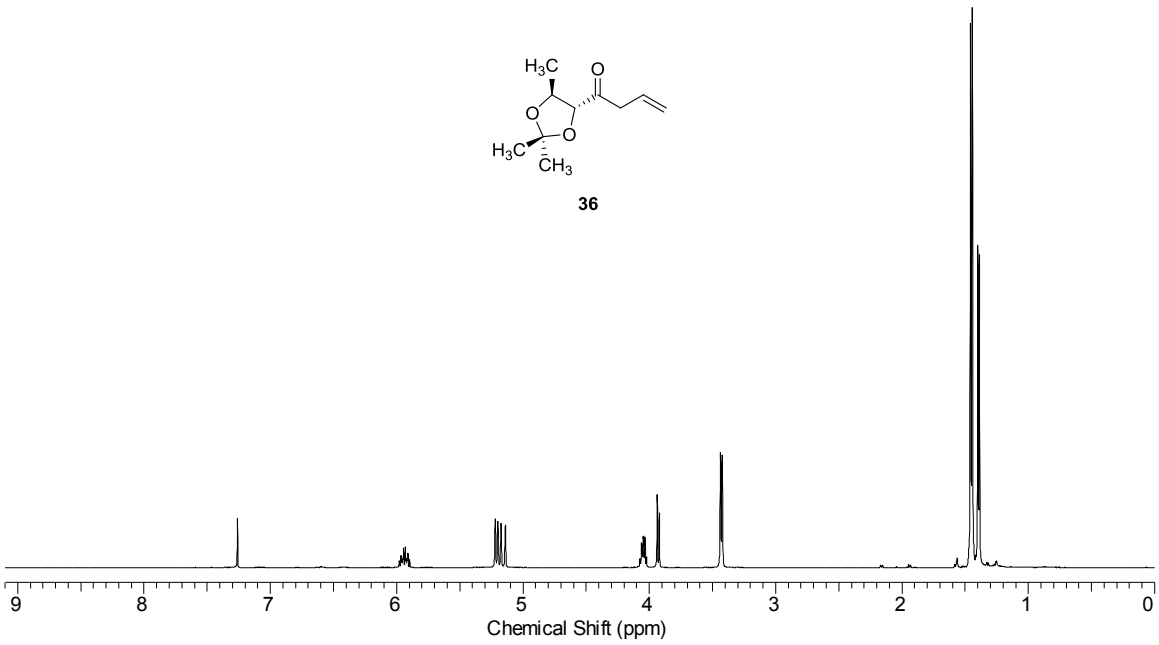
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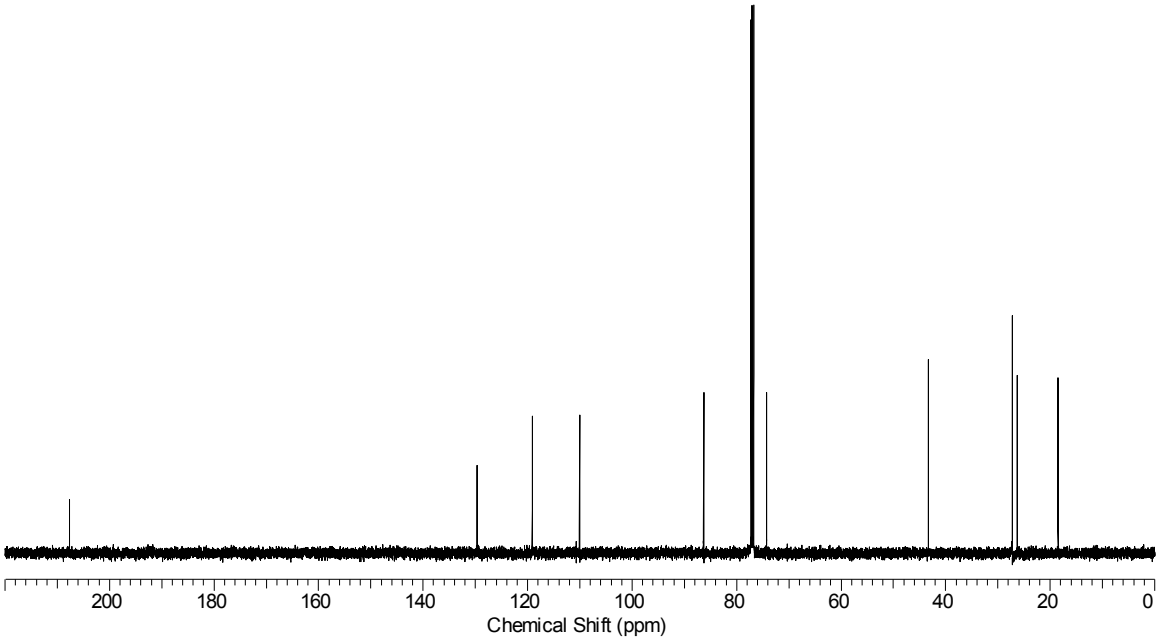
4
3
2
1
0
Chemical Shift (ppm)

**36**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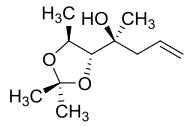
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H3C
CH3

37


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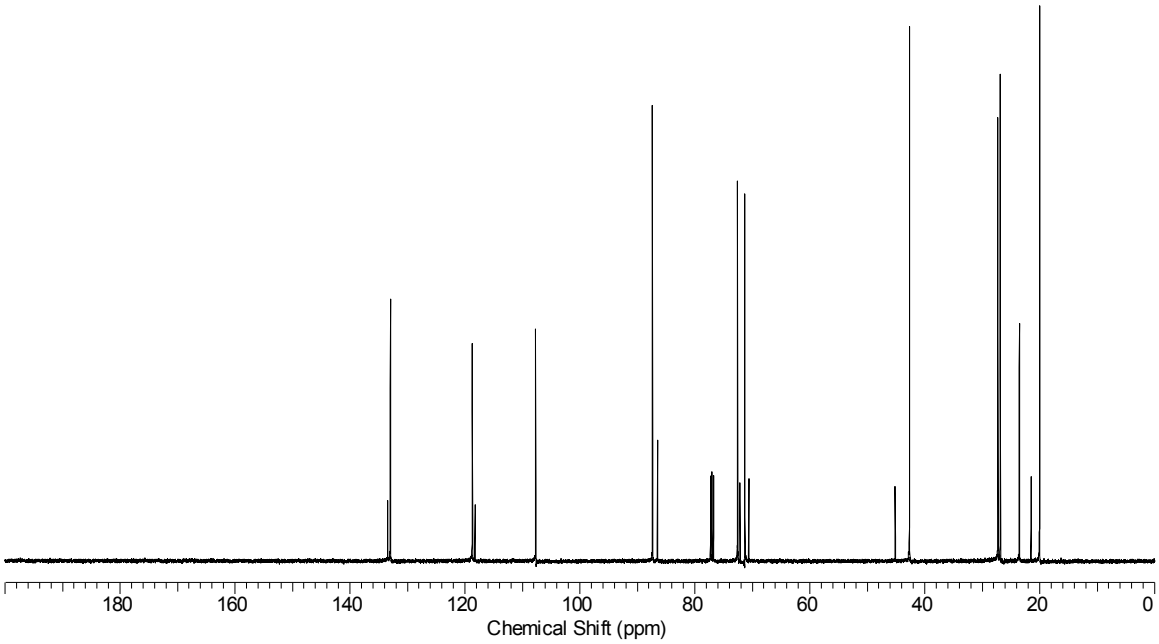
5
4
3
2
1
0
Chemical Shift (ppm)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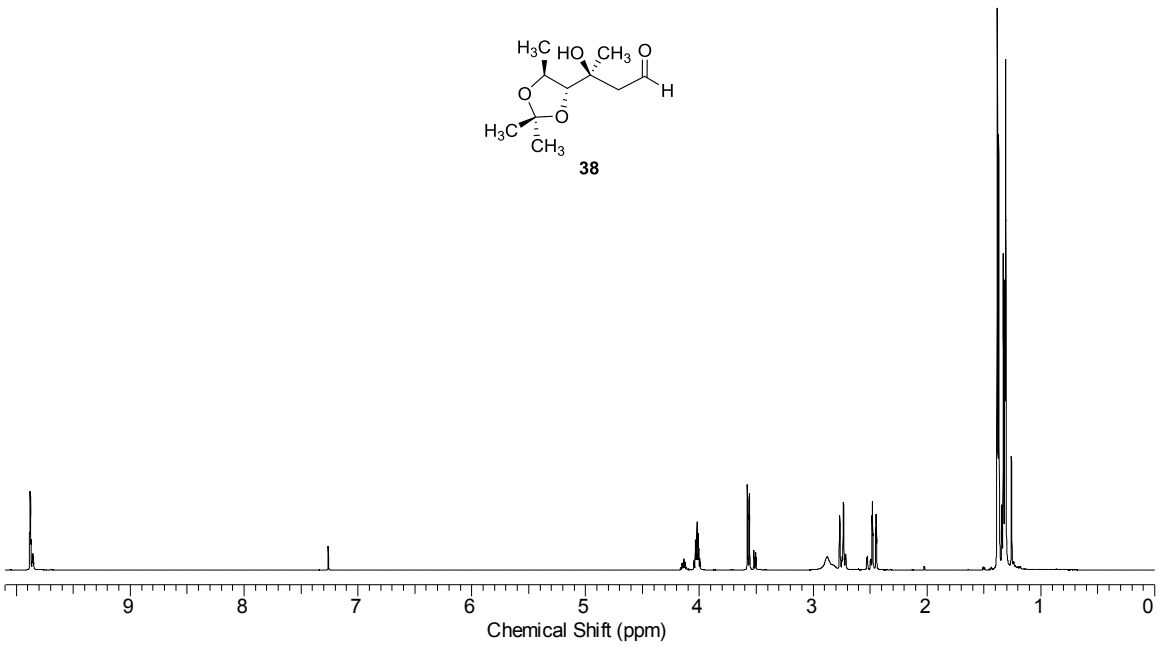
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H
O
H3C
CH3
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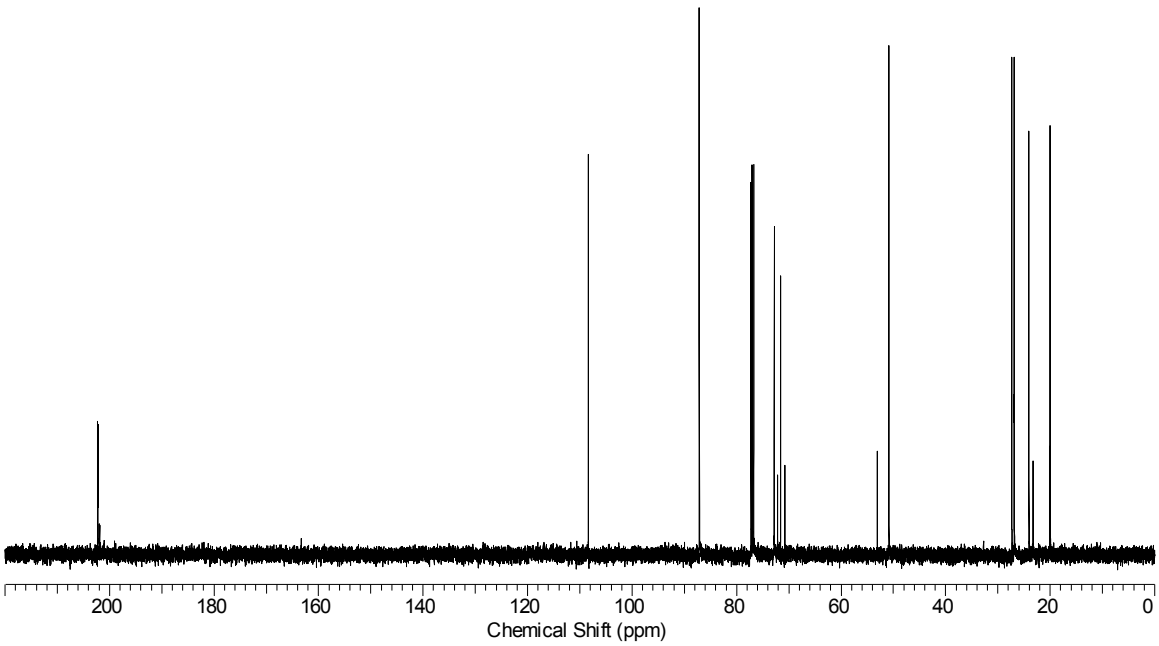
7
6
5
4
3
2
1
0
Chemical Shift (ppm)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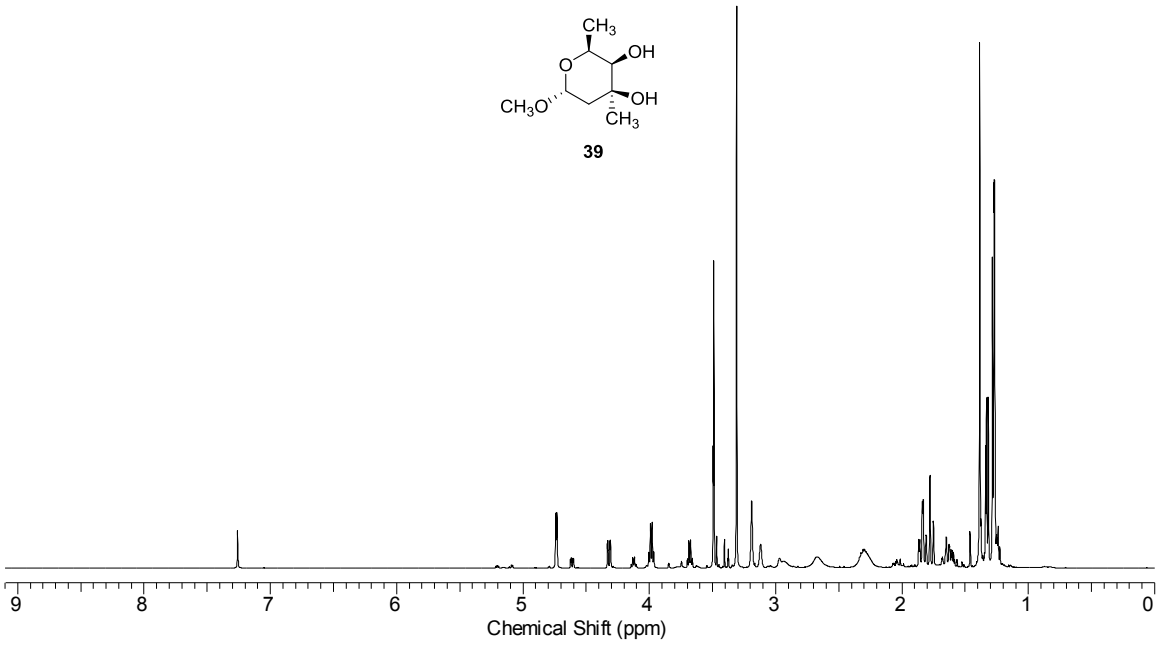
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7
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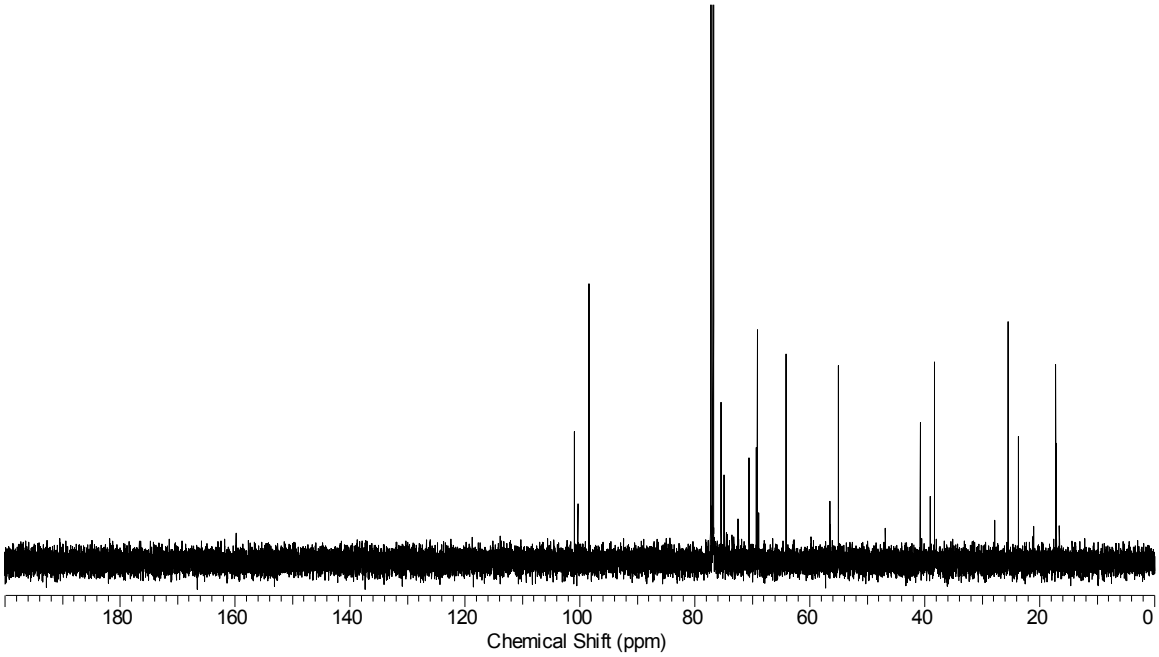
1
0
Chemical Shift (ppm)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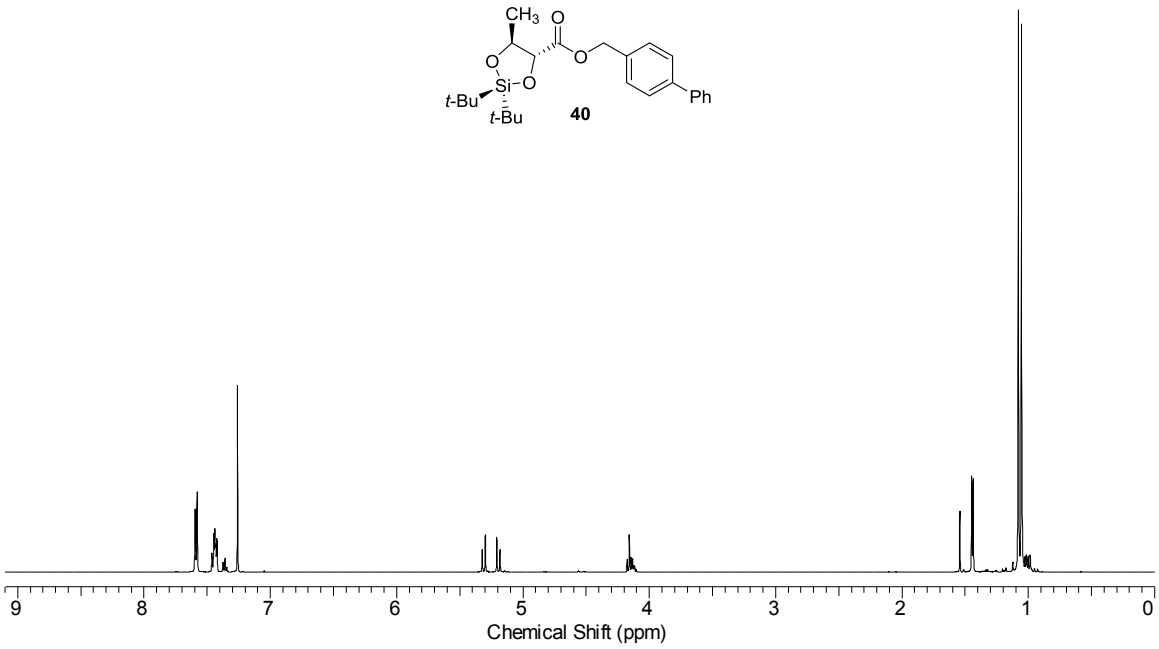
Chemical Shift (ppm)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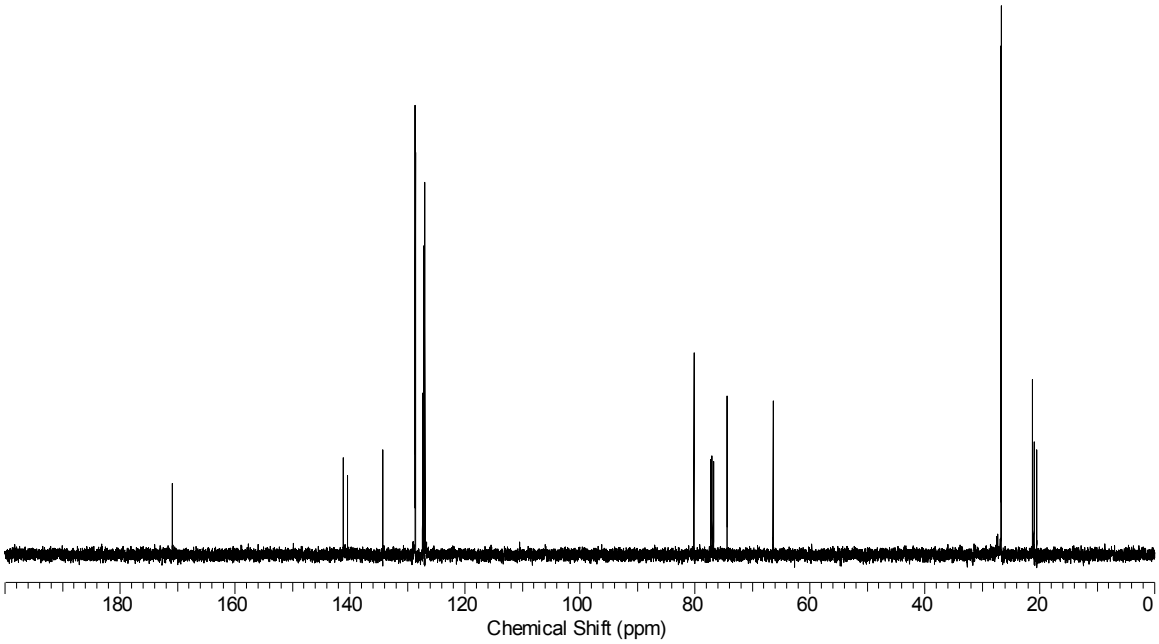
Chemical Shift (ppm)

**40**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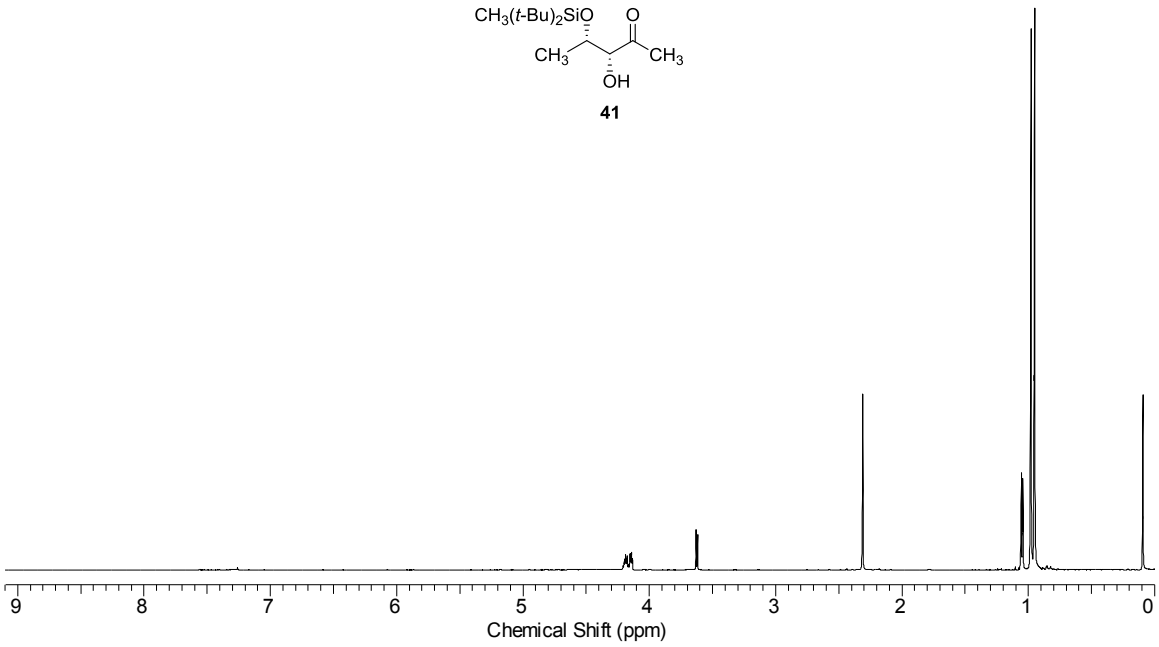
Chemical Shift (ppm)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CH3
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1
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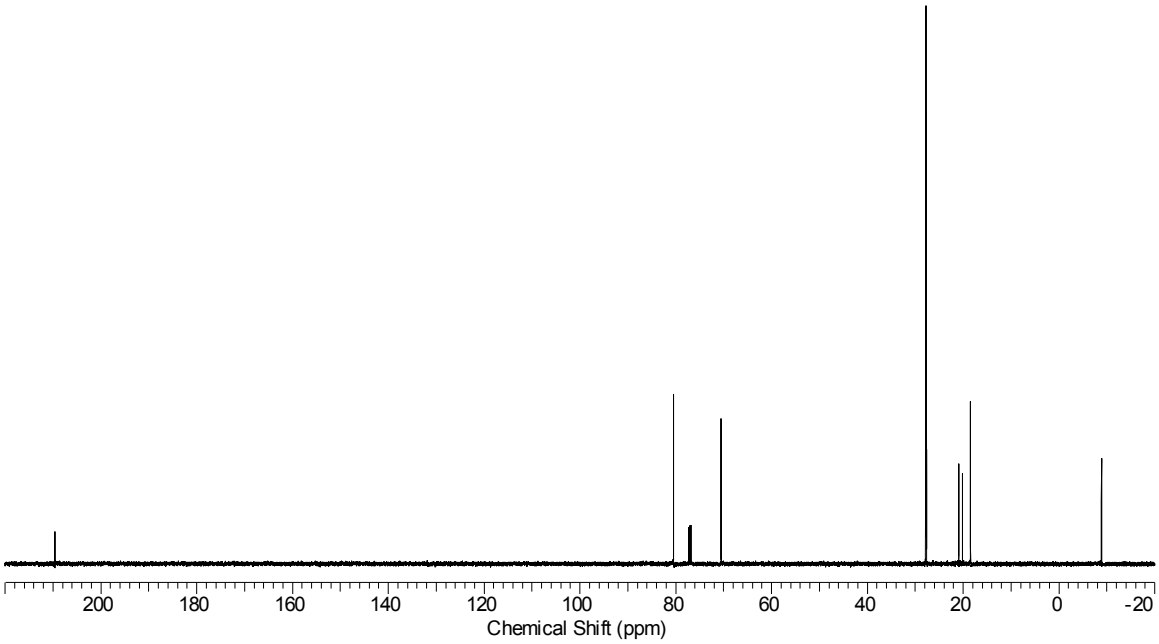
Chemical Shift (ppm)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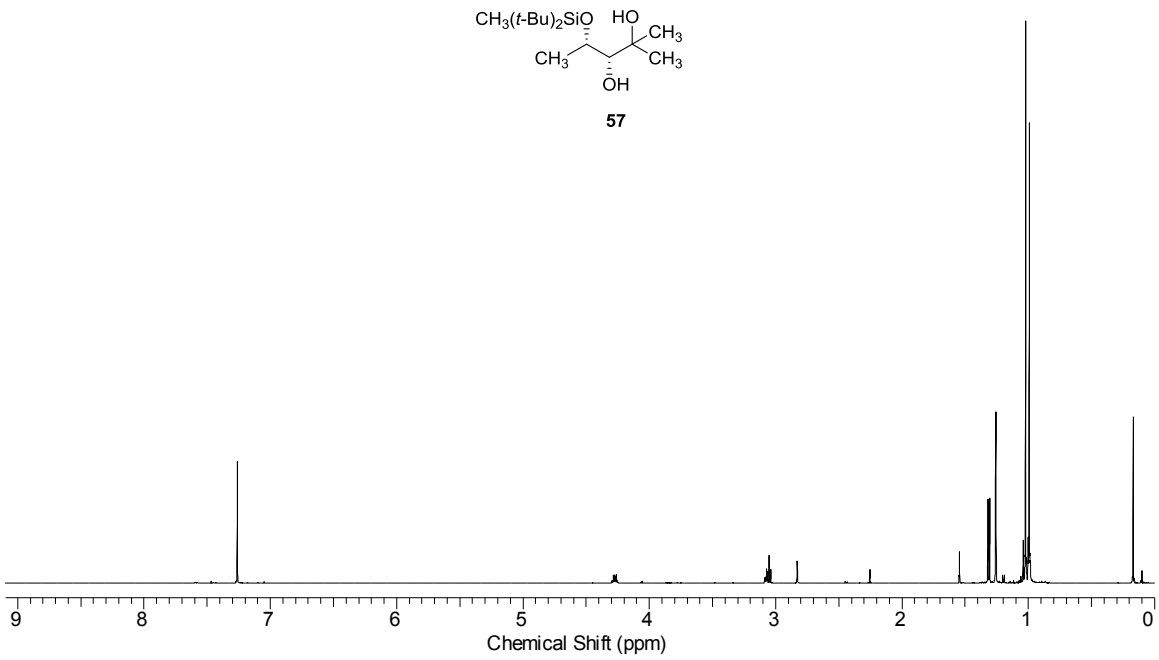
60
40
20
0
-20
Chemical Shift (ppm)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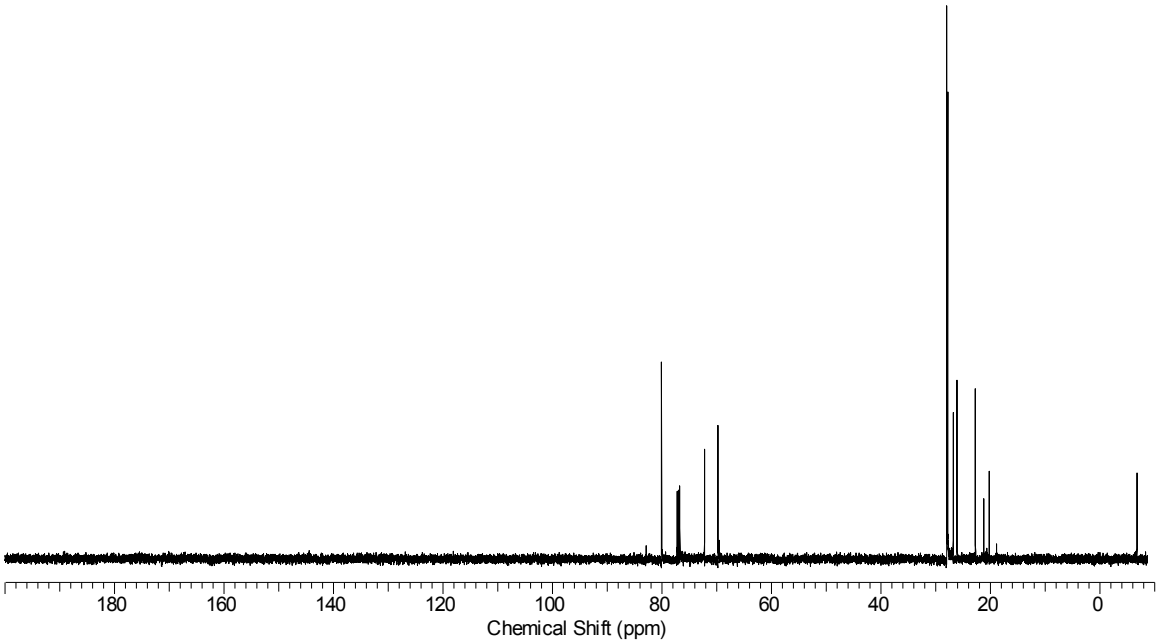
1
0
Chemical Shift (ppm)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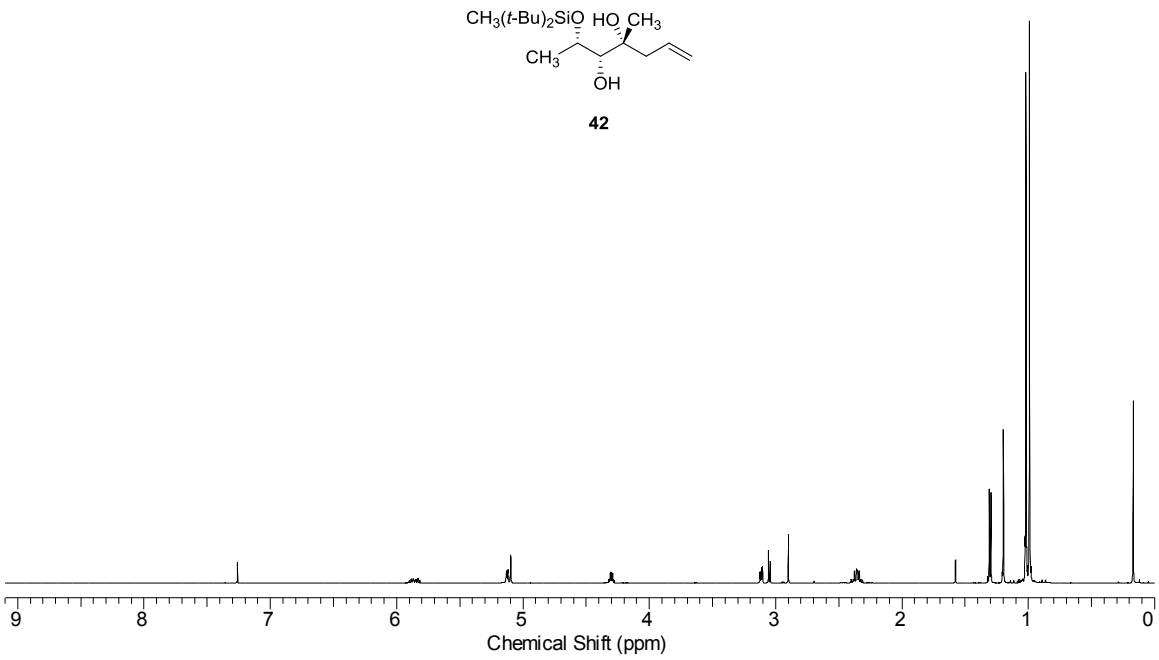
Chemical Shift (ppm)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1
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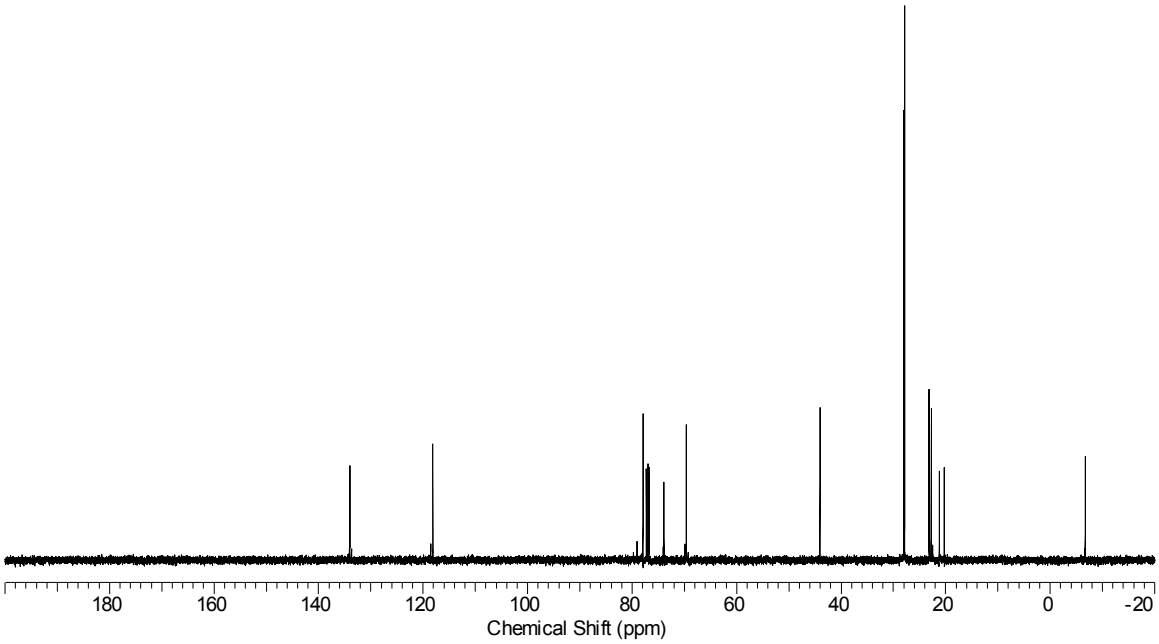
Chemical Shift (ppm)


180
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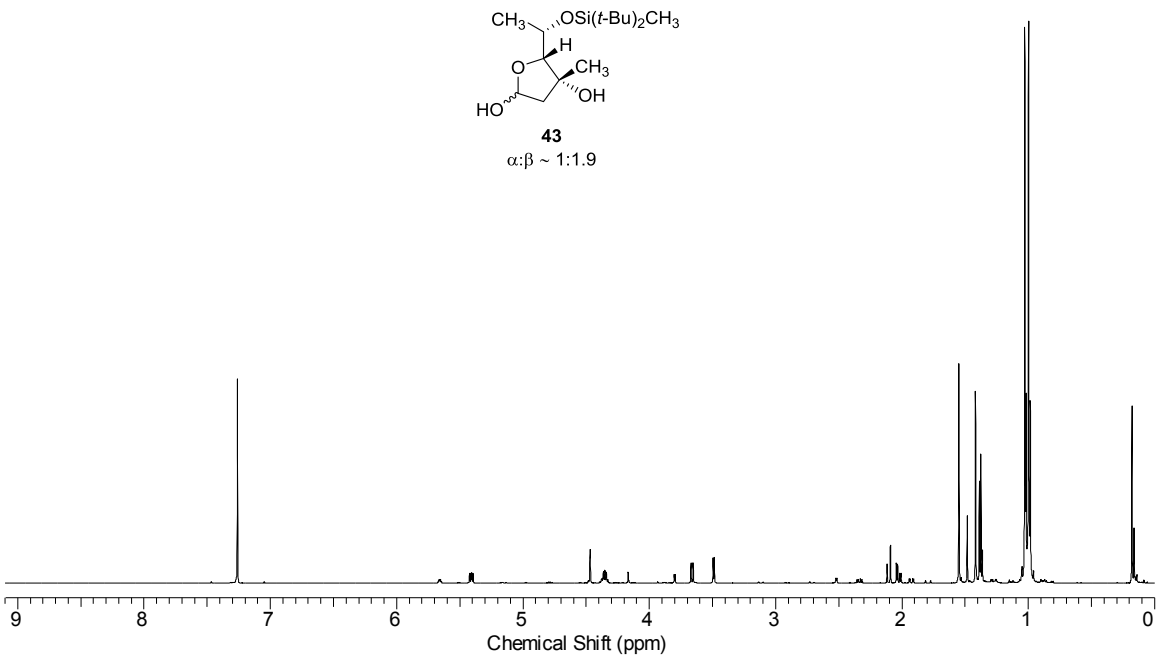
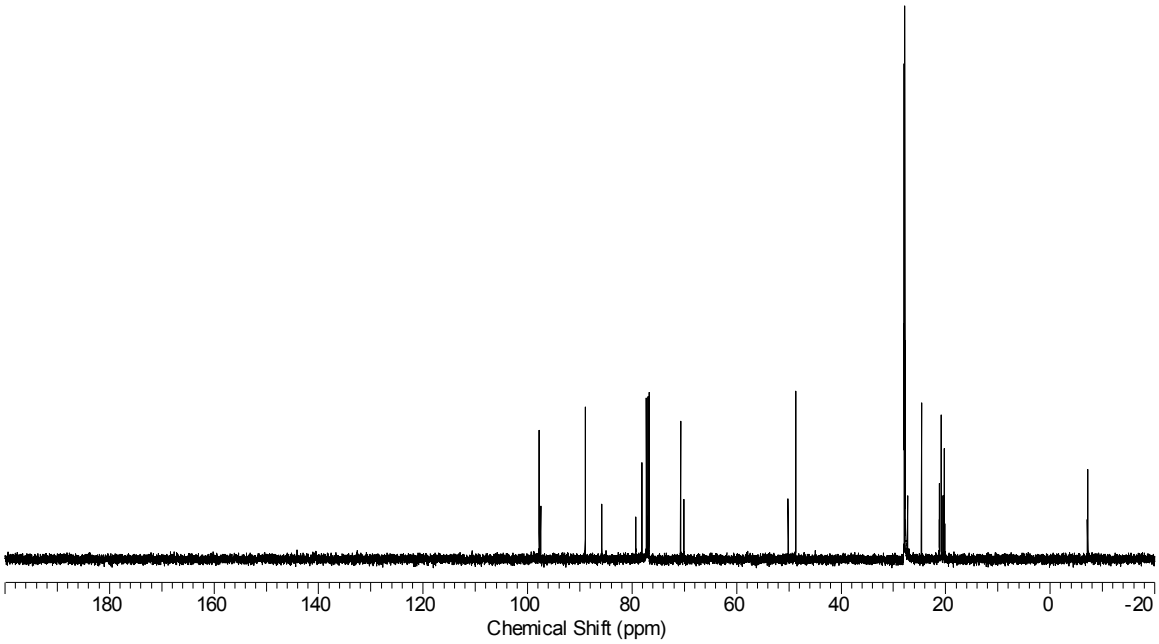
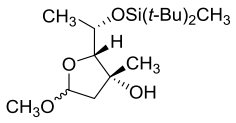
60
40
20
0
-20
Chemical Shift (ppm)


CH3
OSi(t-Bu)2CH3
H
O
CH3
OH
HO
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

**43**

α:β ~ 1:1.9

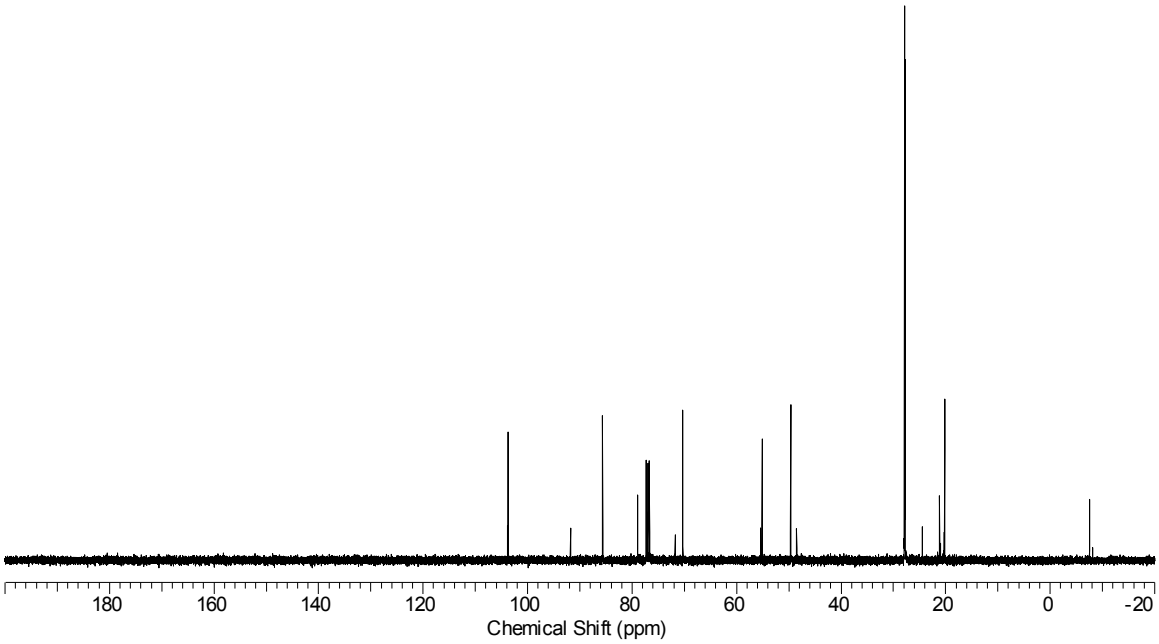

180
160
140
120
100
80
60
40
20
0
-20
Chemical Shift (ppm)

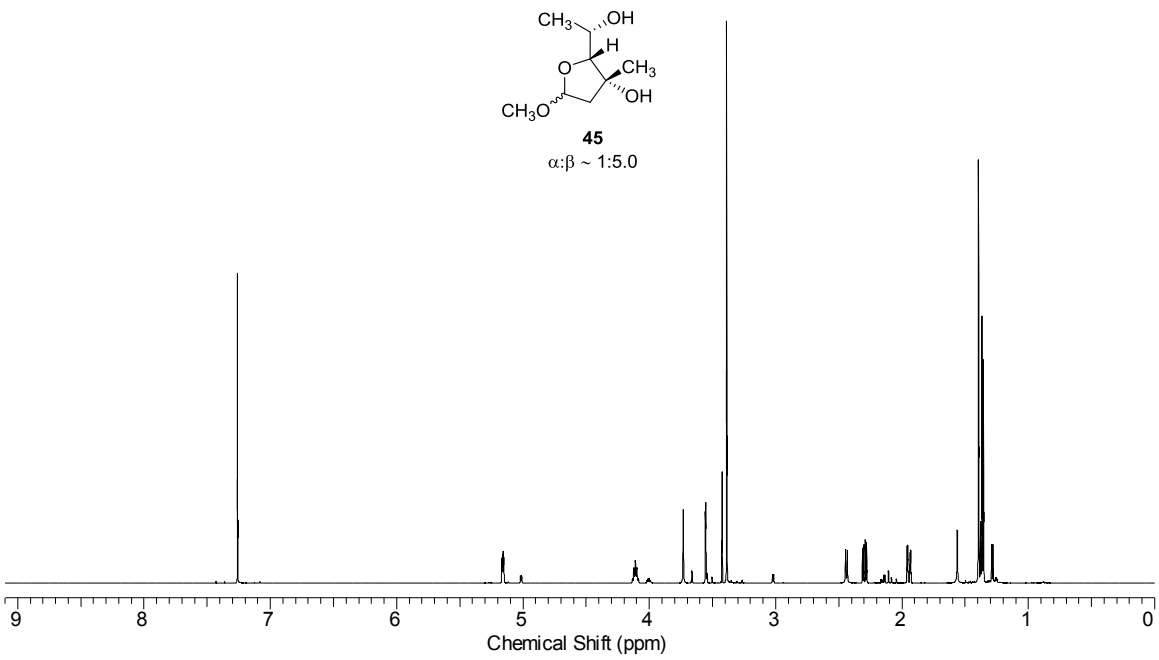

CH3
OSi(t-Bu)2CH3
H
O
CH3
OH
CH3O

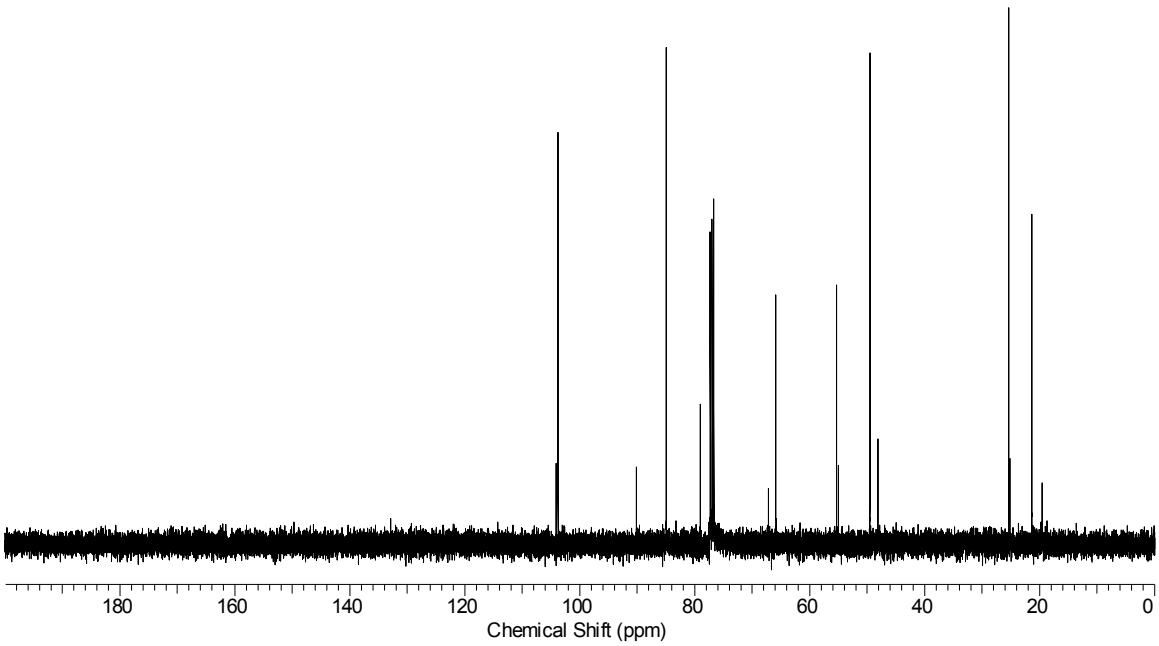
**44**

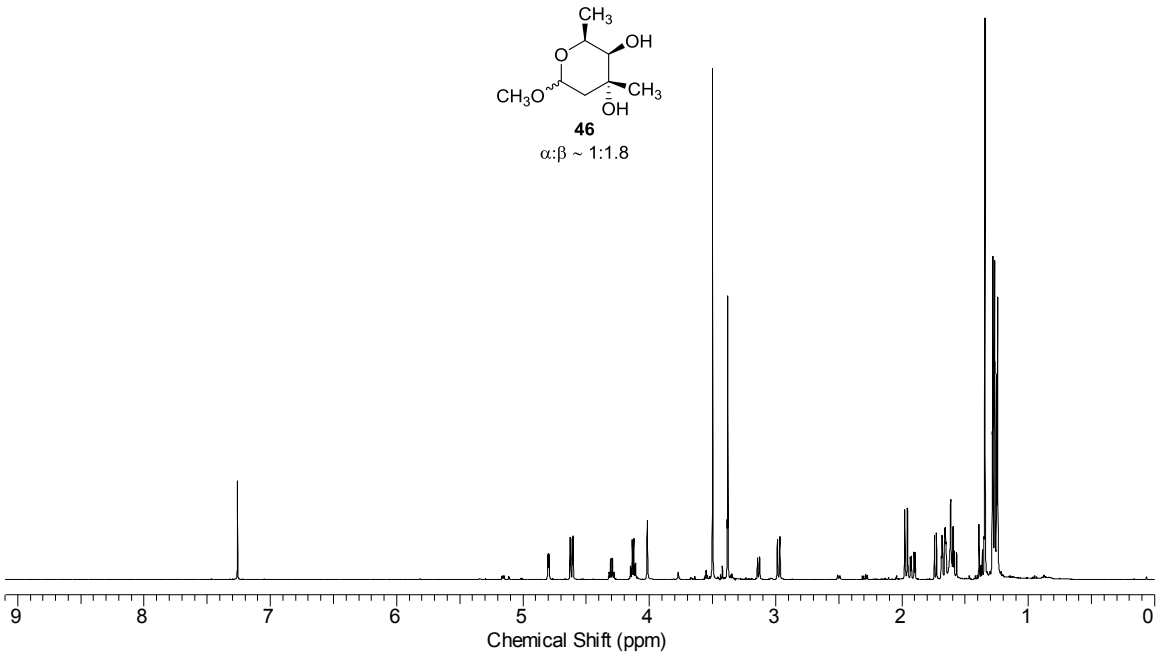
α:β ~ 1:4.8

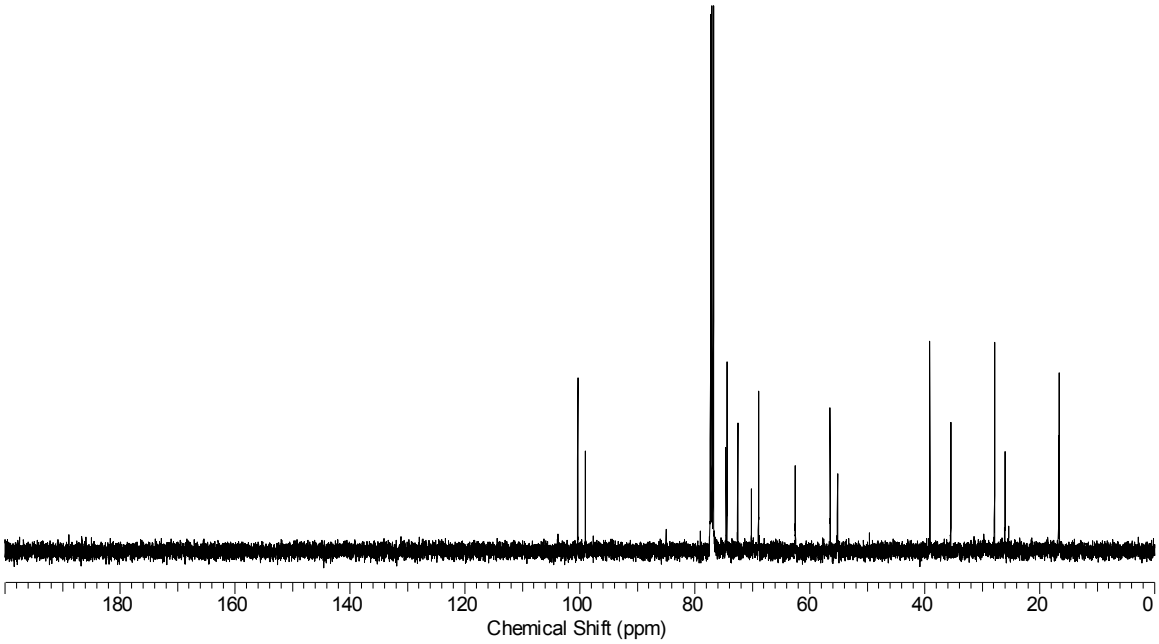

9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

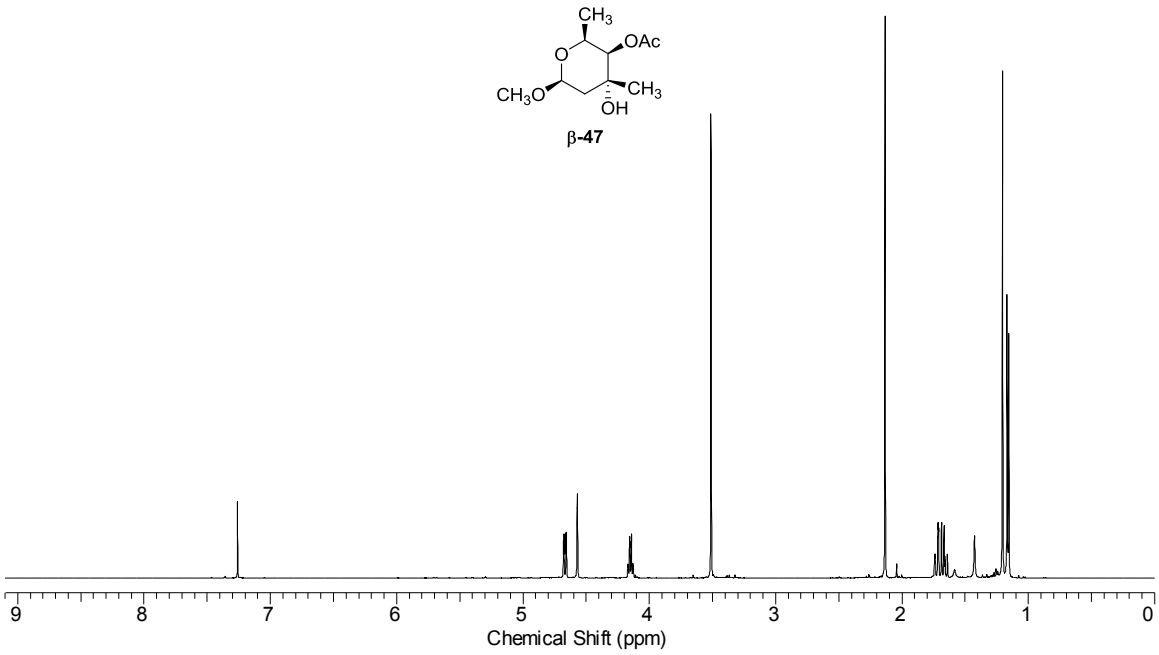

180
160
140
120
100
80
60
40
20
0
-20
Chemical Shift (ppm)


CH3
OH
H
O
CH3
OH
CH3O
45
α:β ~ 1:5.0
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

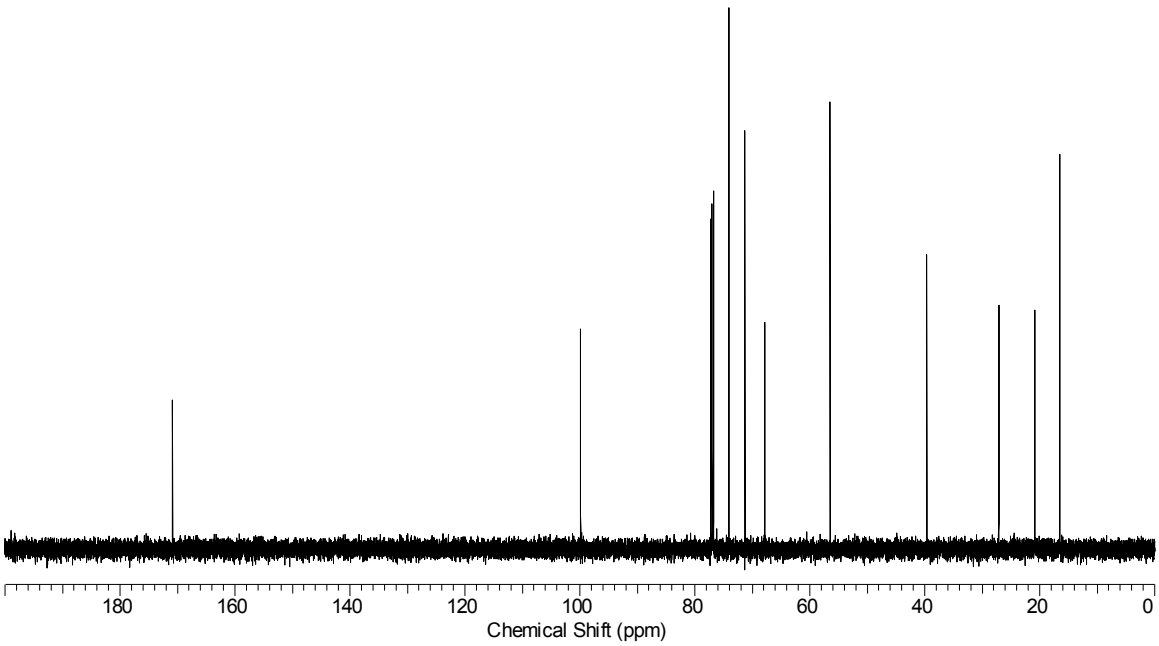

180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

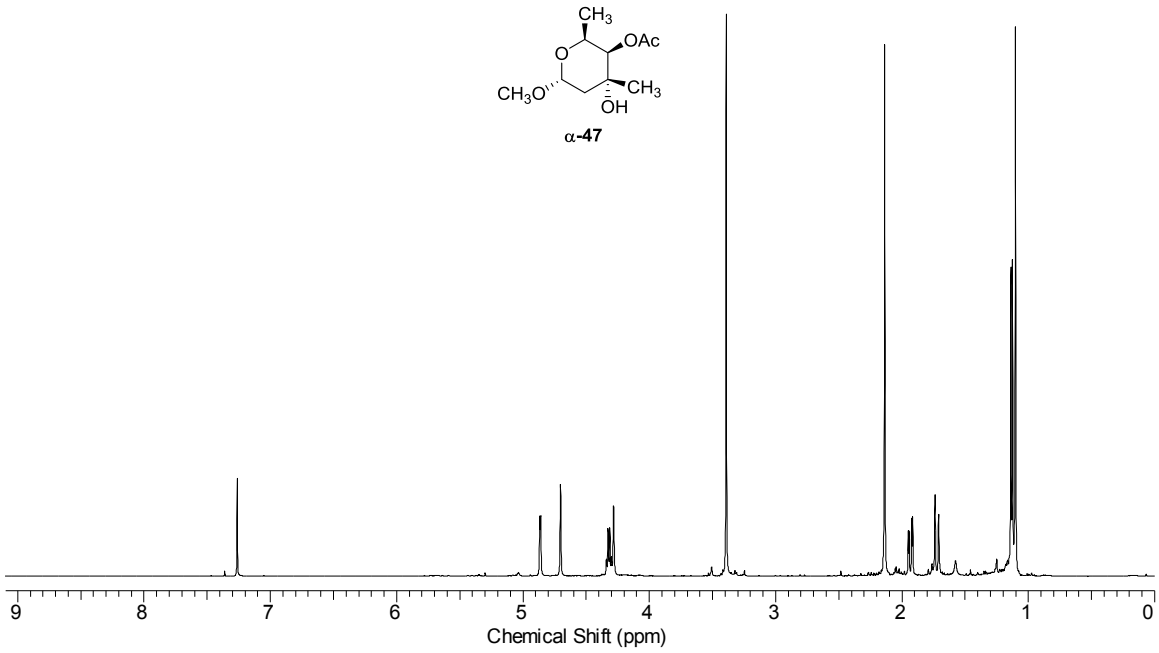

CH3
OH
O
CH3O
CH3
OH
46
α:β ~ 1:1.8
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)


180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

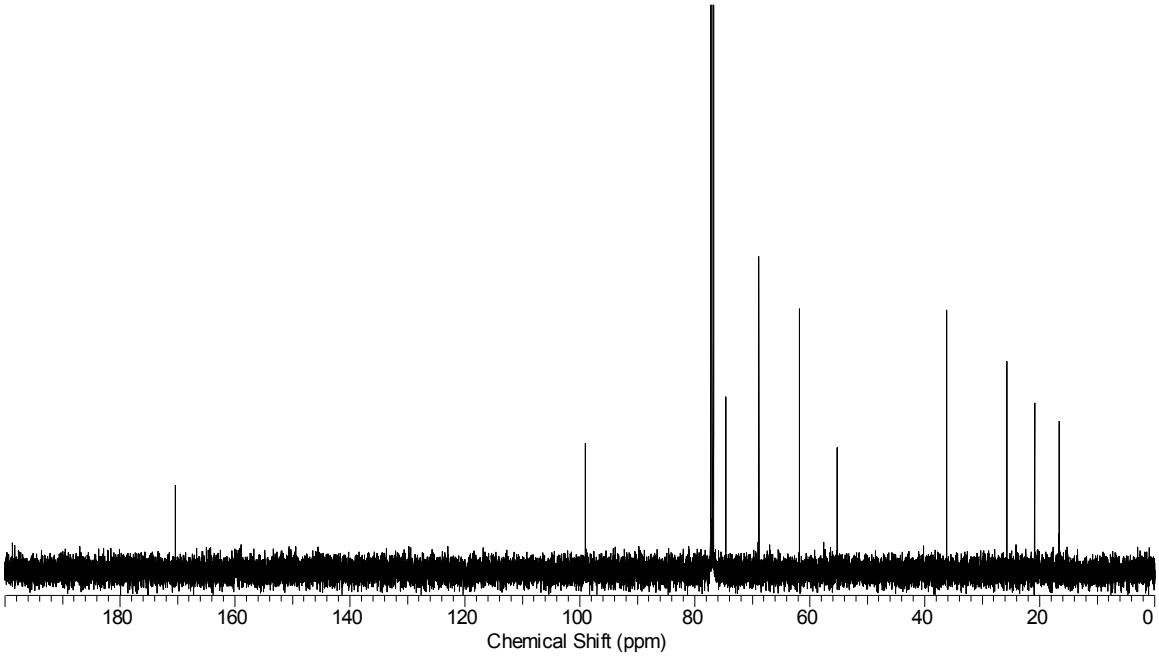

CH3
OAc
O
CH3O
CH3
OH
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

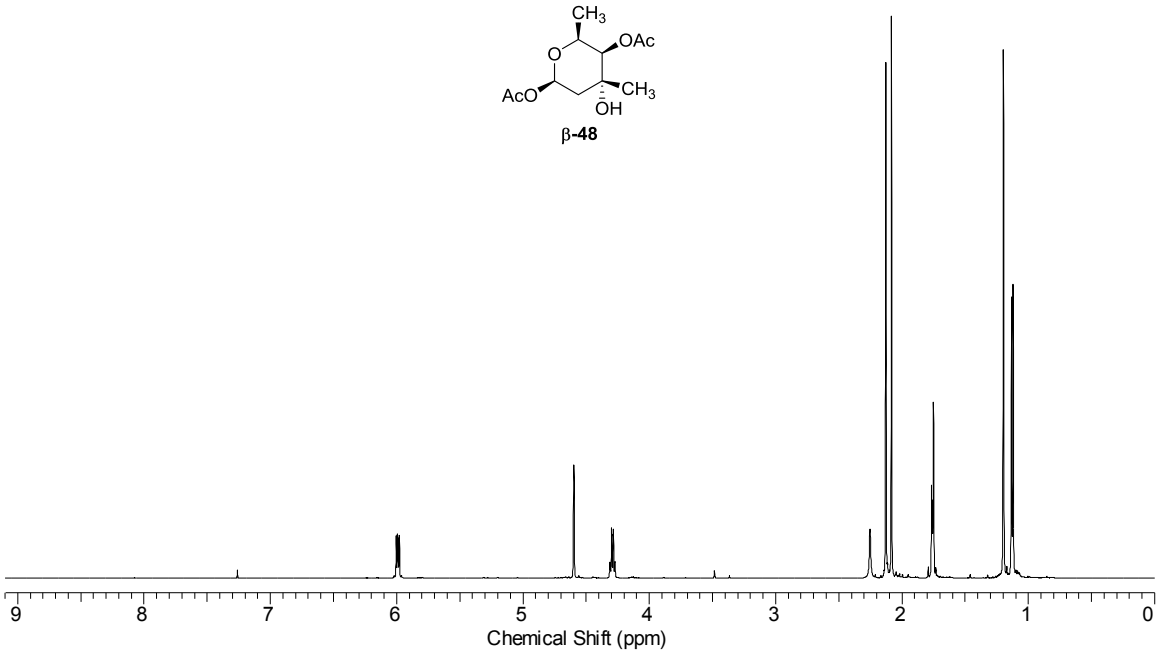
β-**47**

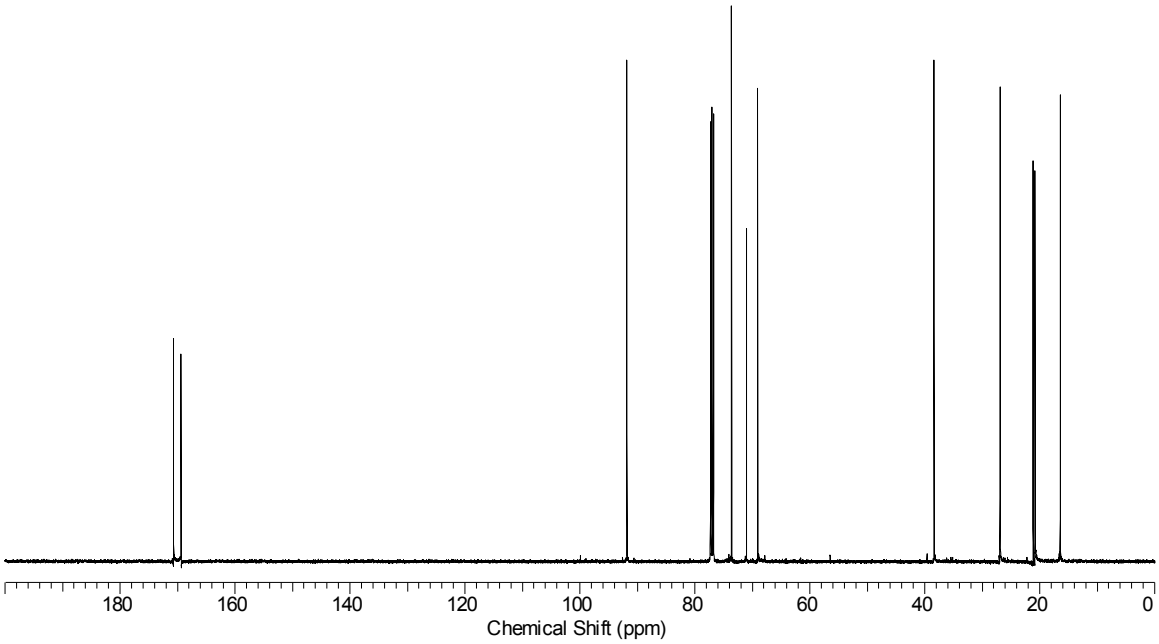

180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

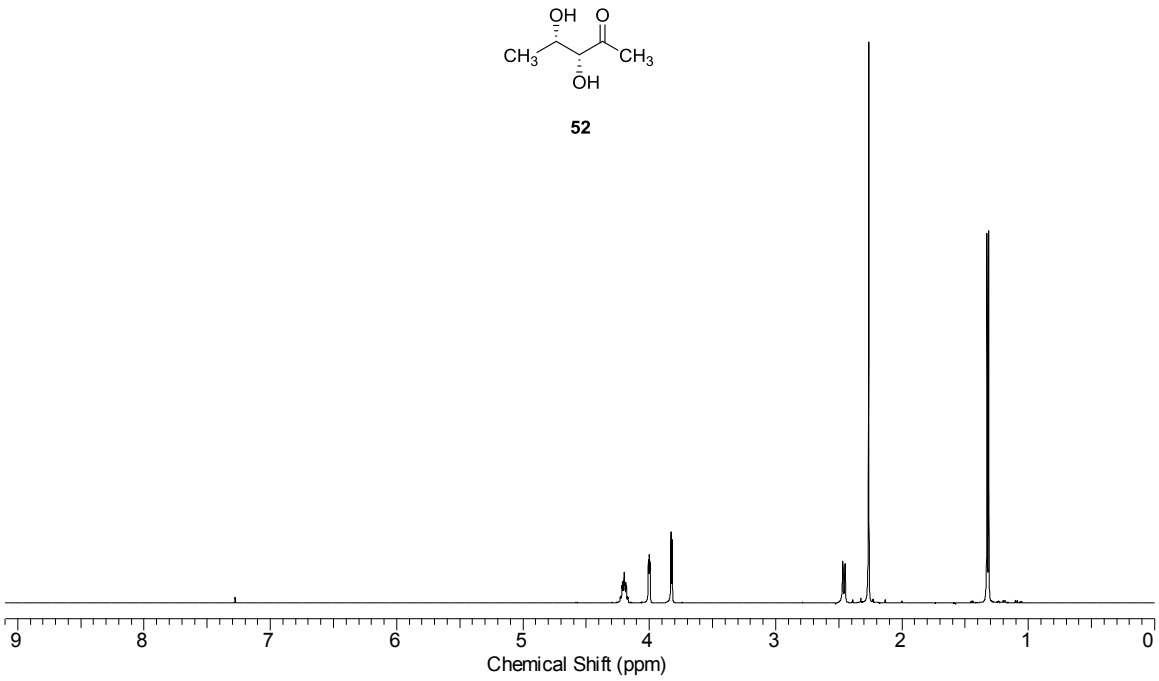

CH3
OAc
O
CH3O
CH3
OH
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

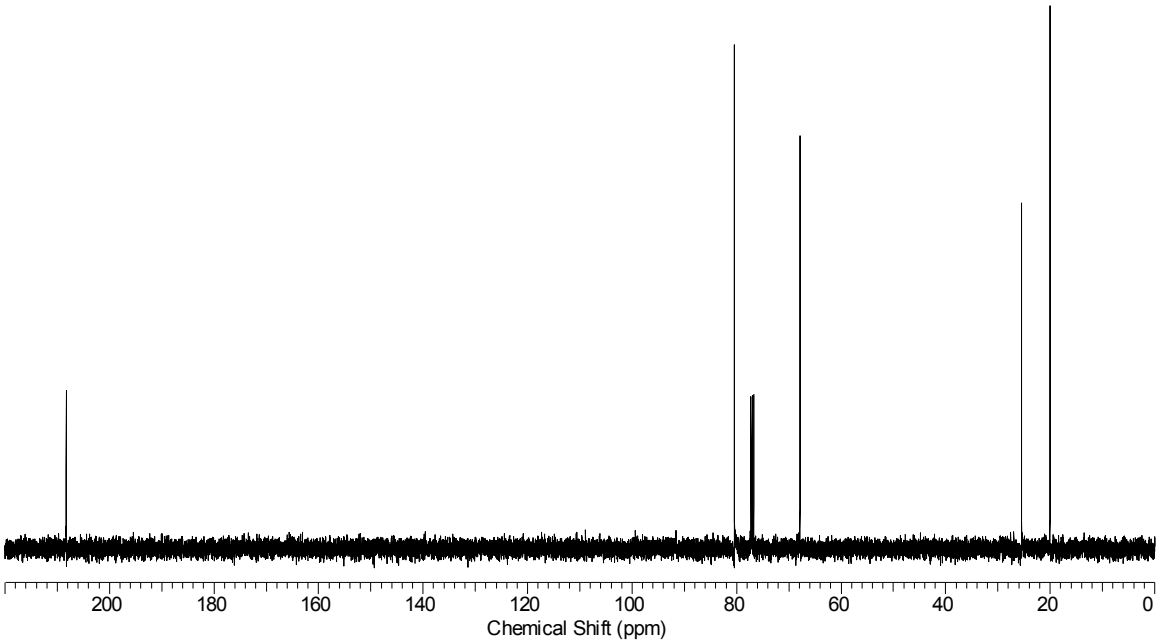
α-**47**

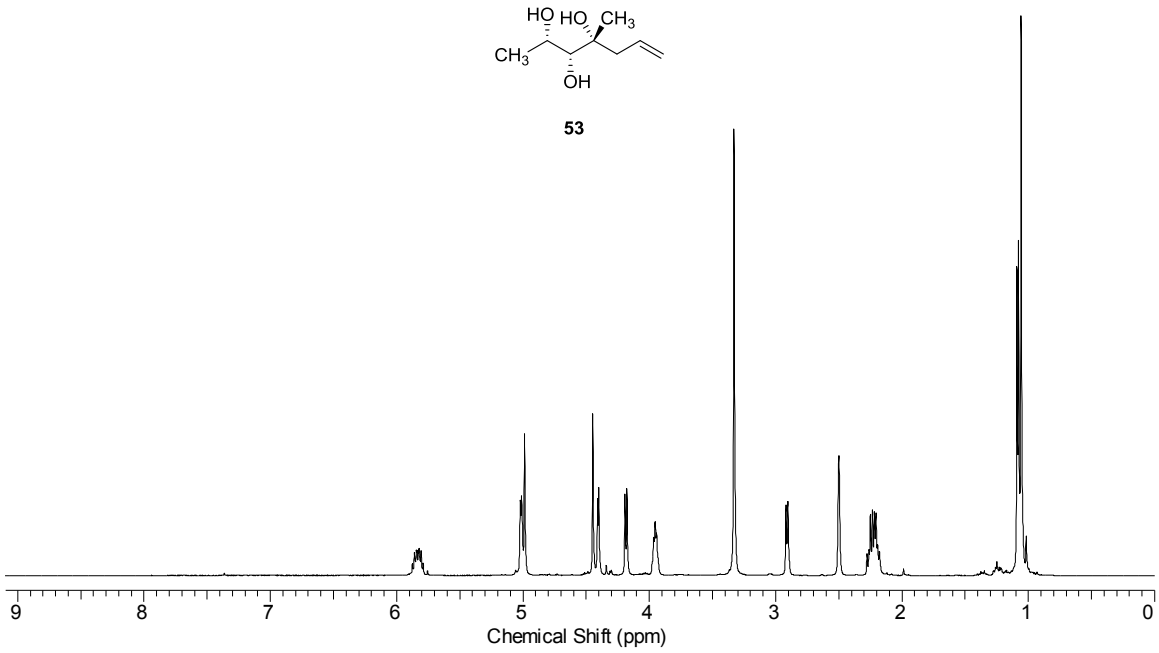

180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

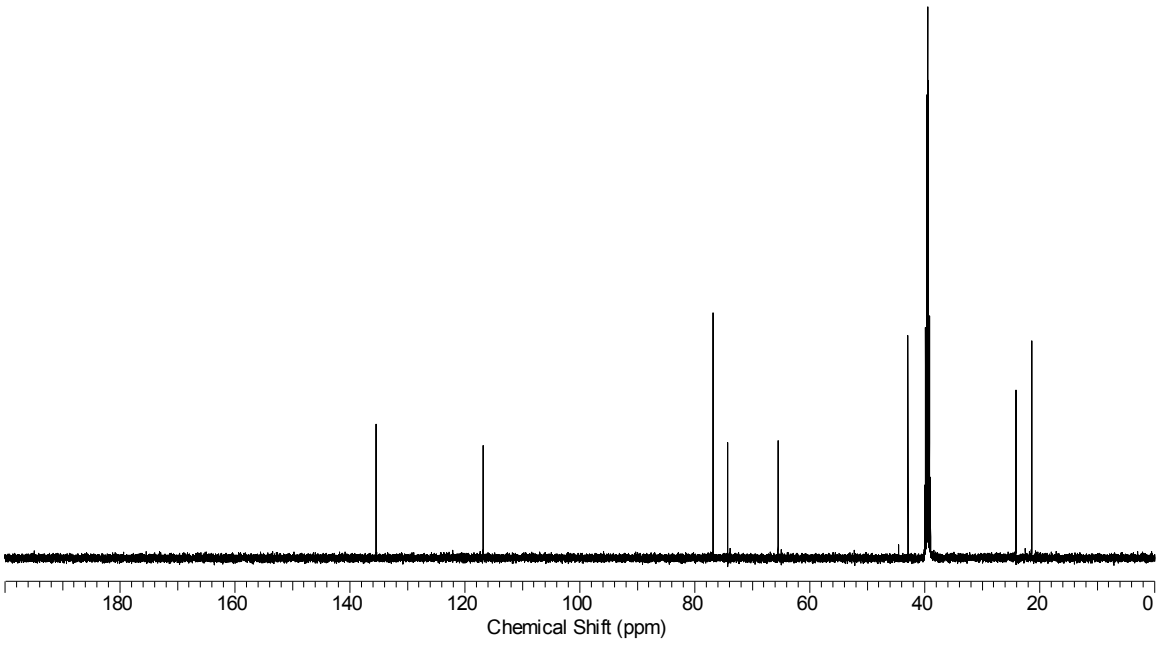

CH3
OAc
O
AcO
CH3
OH
β-48
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

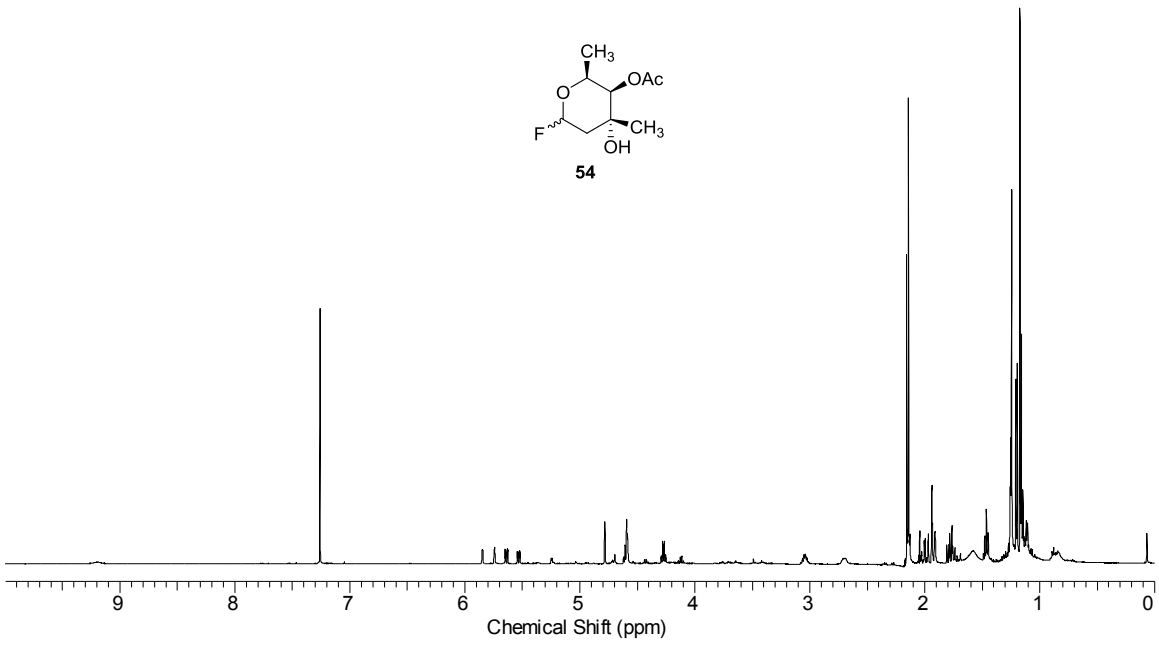

180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

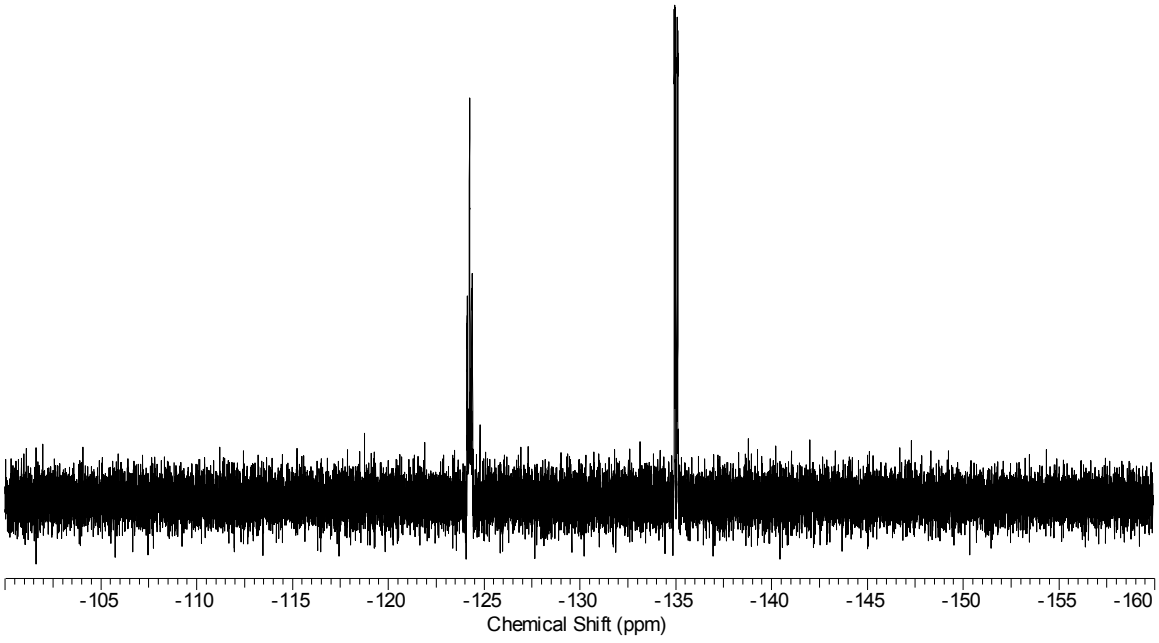

OH
O
CH3
CH3
OH
52
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

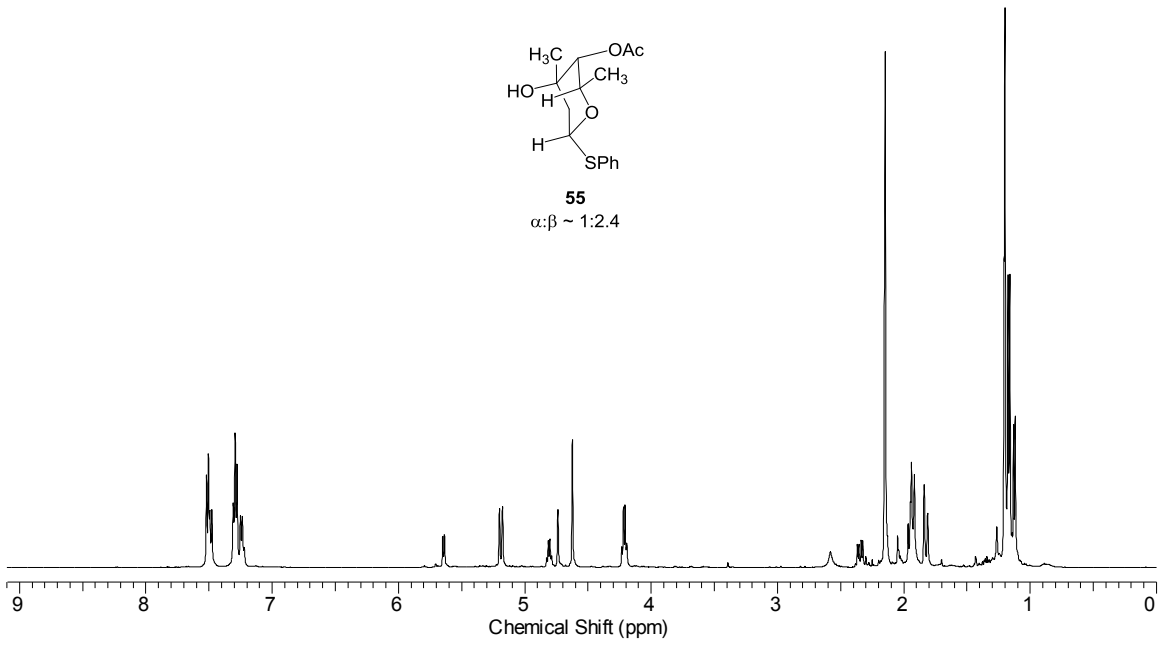

200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

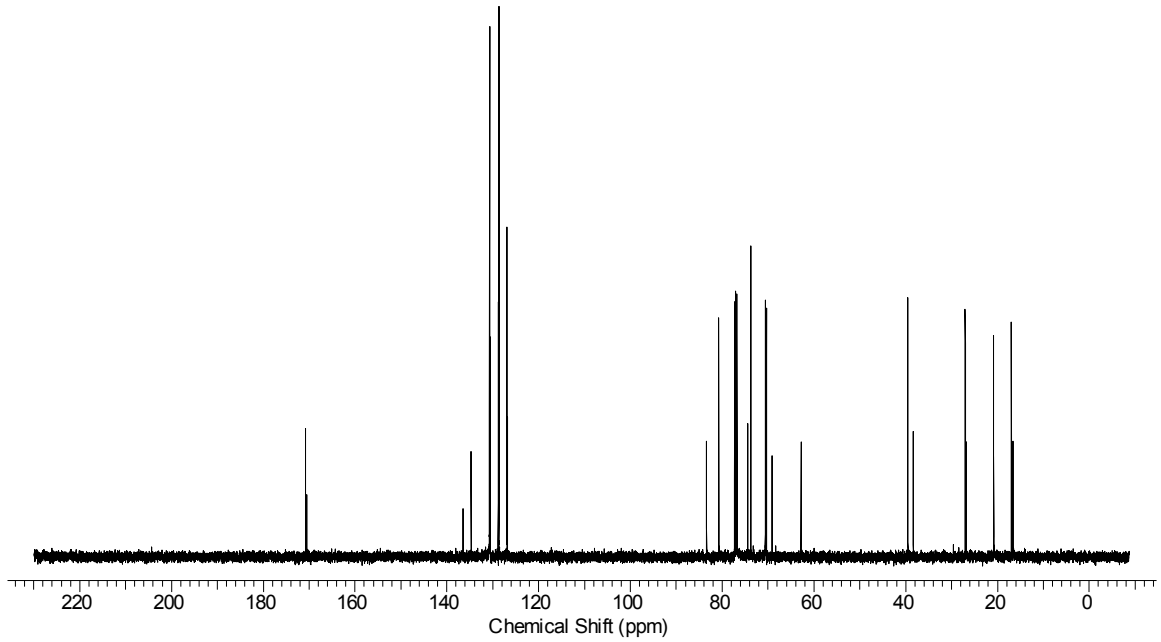

HO
HO
CH3
CH3
OH
53
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

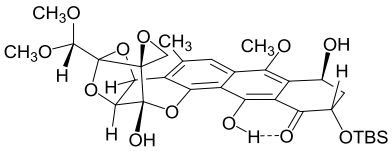

180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)


CH3
OAc
O
F
CH3
OH
54
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)


-105
-110
-115
-120
-125
-130
-135
-140
-145
-150
-155
-160
Chemical Shift (ppm)

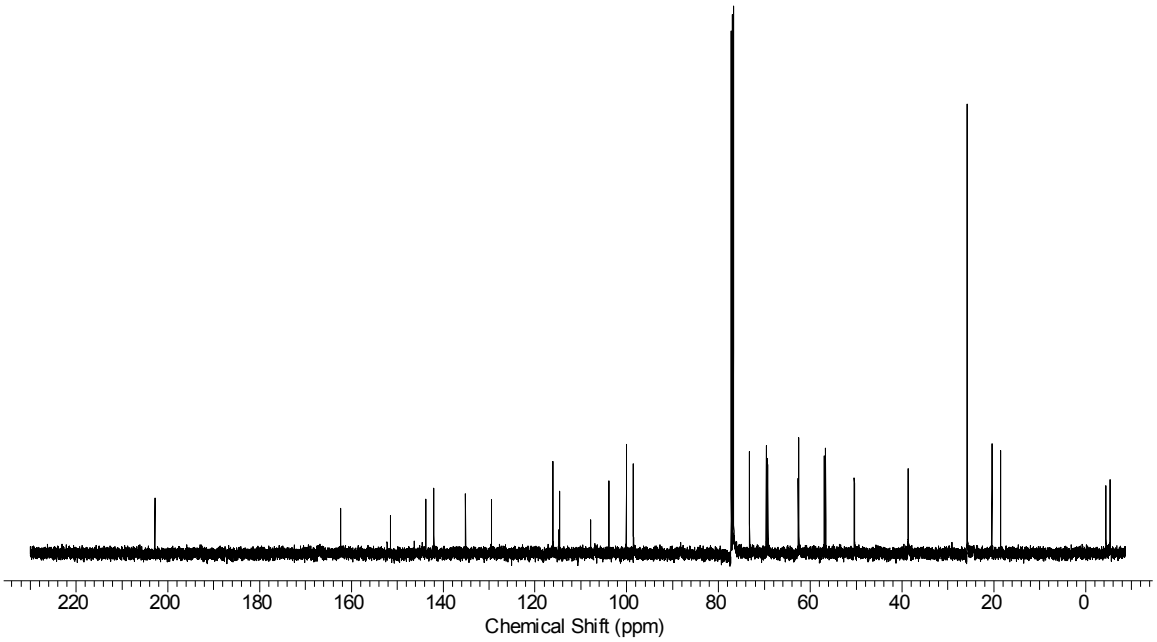

H3C
OAc
CH3
HO
H
O
H
SPh
55
α:β ~ 1:2.4
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

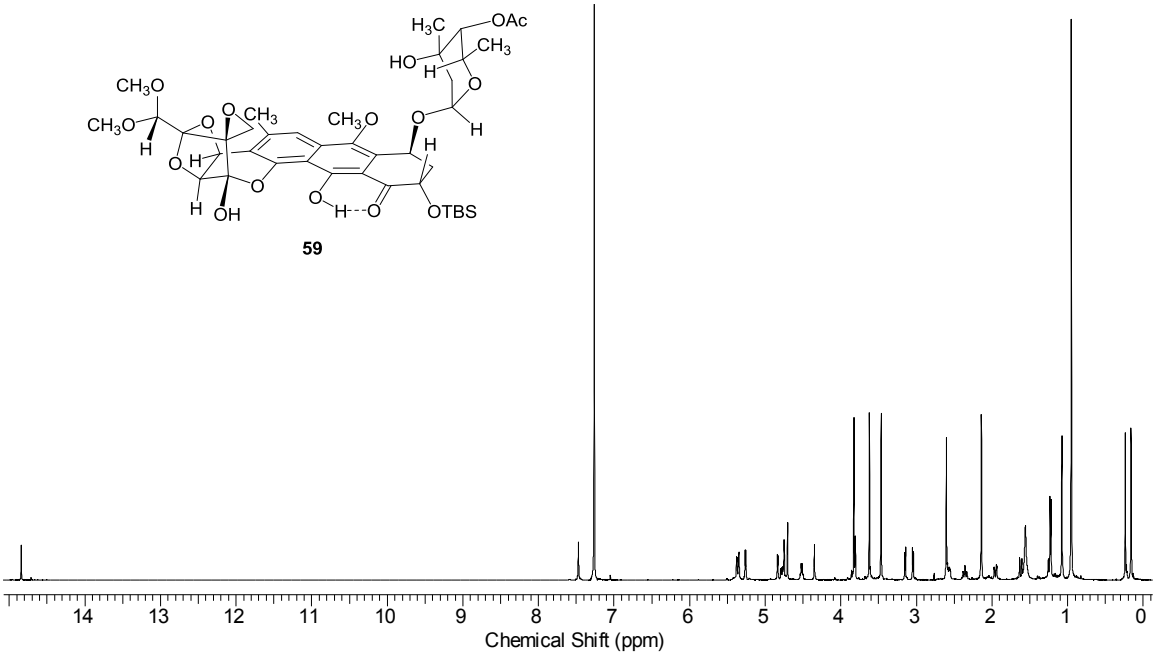

220
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)


CH3O
CH3O
H
O
O
CH3
O
H
H
O
O
H
OH
CH3O
OH
H
O
H···O
OTBS

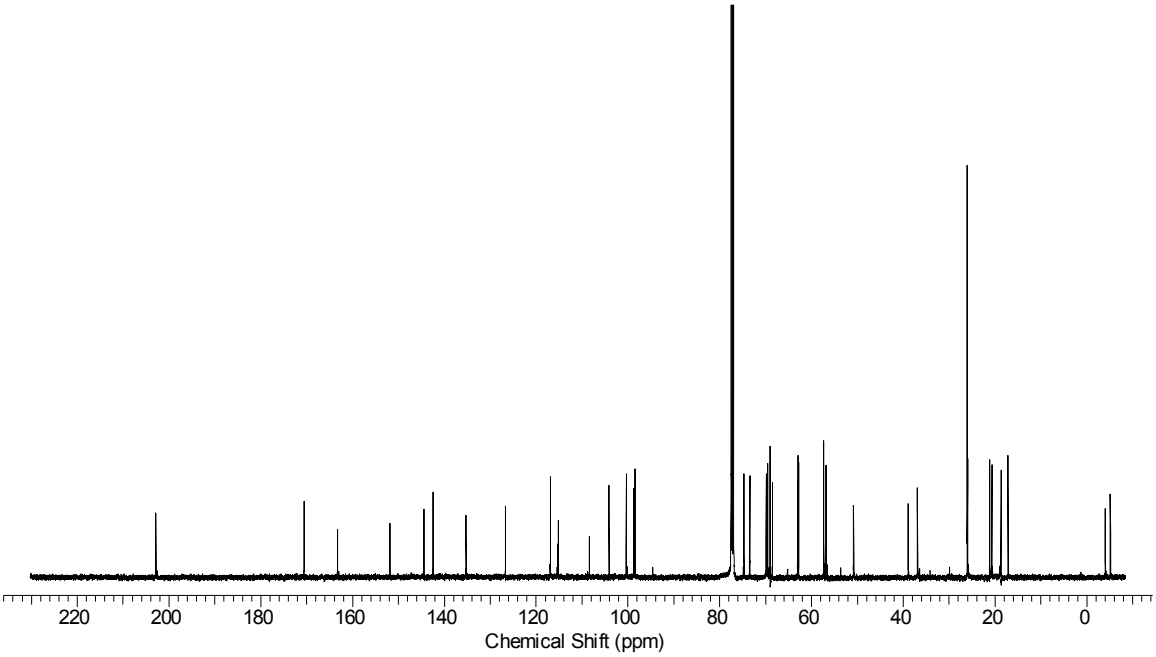
**58**

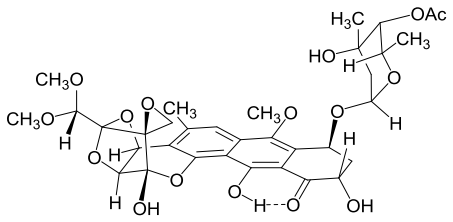

14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)


220
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)


H3C
OAc
CH3
HO
H
O
CH3O
CH3O
H
O
O
CH3
CH3O
O
H
O
H
H
O
H
OH
O
H
O
OTBS
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

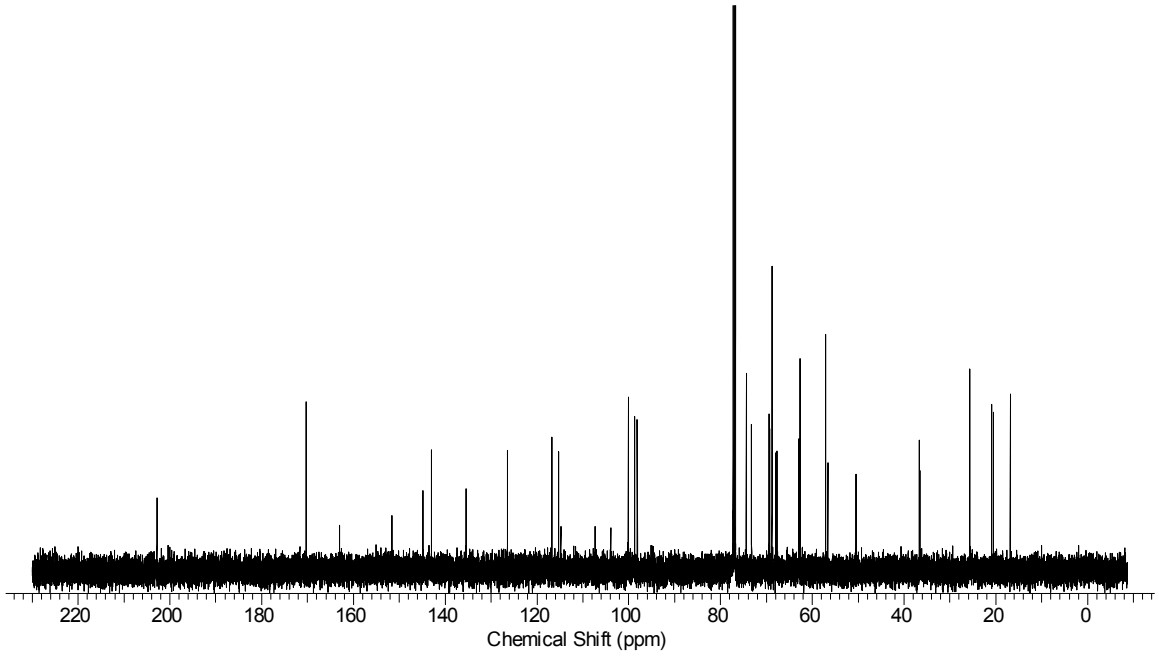
**59**


220
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

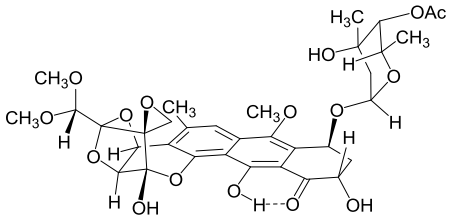

H3C
OAc
CH3
HO
H
O
CH3O
CH3O
O
CH3
CH3O
O
H
H
O
H
O
O
H
OH
O
H---O
OH

DC-45-A1 (**10**)


14
13
12
11
10
9
8
7
6
5
4
3
2
1
Chemical Shift (ppm)


220
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

## Comparison of Natural and Synthetic DC-45-A1 (10)




DC-45-A1 (##)

Comparison of $^{1}$H NMR Spectral Data of Natural and Synthetic DC-45-A1 (**10**)

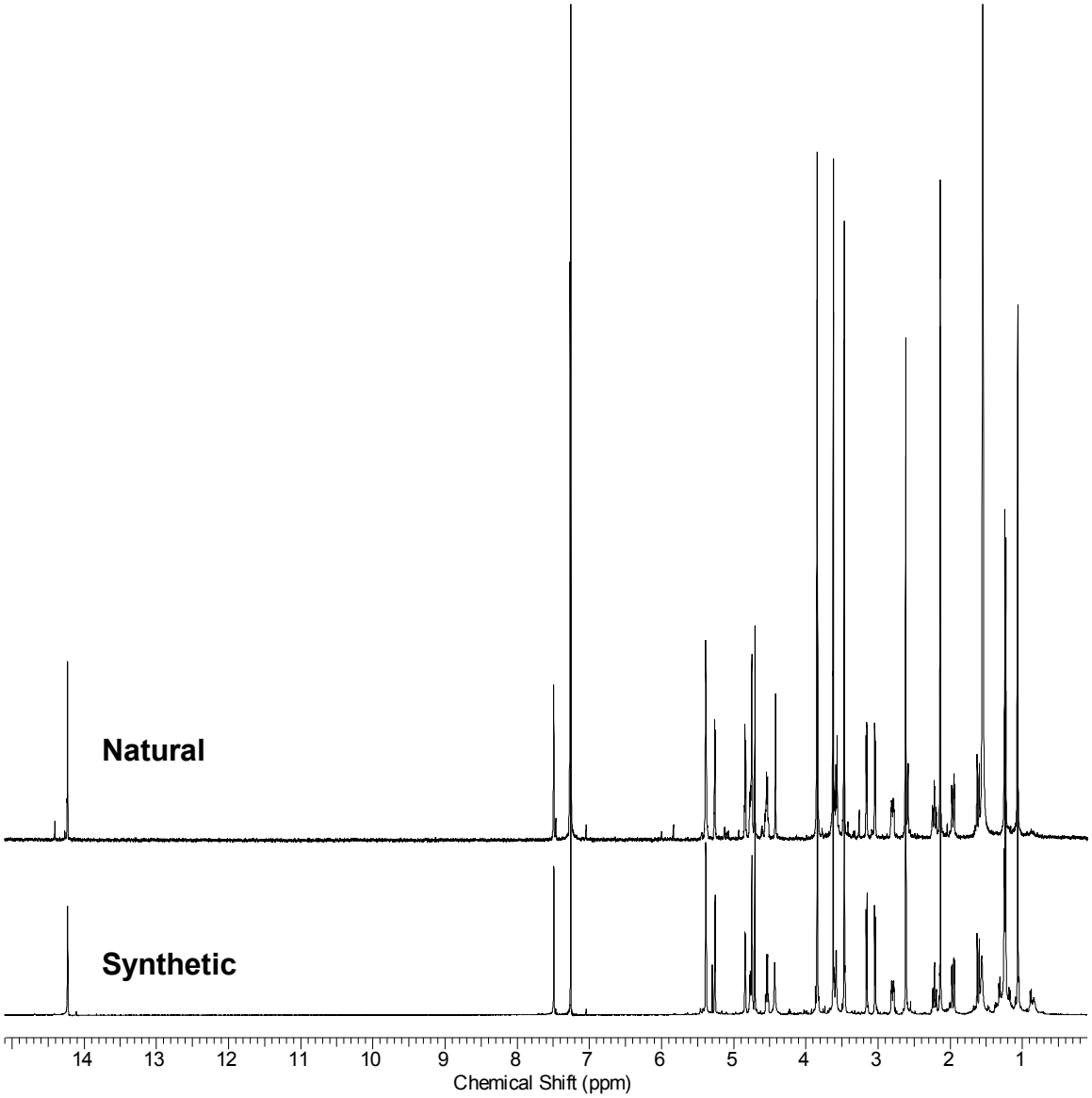
| **Natural**[3] ($CDCl_3$) | **Synthetic** 500 MHz ($CDCl_3$) |
|---|---|
| 1.07 (s, 3H) | 1.07 (s, 3H) |
| 1.24 (d, *J* = 6.4 Hz, 2H) | 1.24 (d, *J* = 6.2 Hz, 3H) |
| **1.50–2.50** | 1.61 (d, *J* = 14.3 Hz, 1H) |
| – | 1.96 (dd, *J* = 14.3, 4.0 Hz, 1H) |
| 2.14 (s, 3H) | 2.14 (s, 3H) |
| – | 2.22 (dt, , *J* = 13.2, 2.6 Hz, 1H) |
| 2.60 (s, 3H) | 2.62 (s, 3H) |
| – | 2.83–2.77 (m, 1H) |
| 3.02 (d, *J* = 5.4 Hz, 1H) | 3.04 (d, *J* = 5.5 Hz, 1H) |
| 3.13 (d, *J* = 5.4 Hz, 1H) | 3.16 (d, *J* = 5.1 Hz, 1H) |
| 3.46 (s, 3H) | 3.47 (s, 3H) |
| – | 3.58 (br, 1H) |
| 3.63 (s, 3H) | 3.62 (s, 3H) |
| 3.84 (s, 3H) | 3.84 (s, 3H) |
| **4.50–5.50** | 4.43 (br, 1H) |
| – | 4.54 (q, *J* = 6.2 Hz, 1H) |
| – | 4.71 (s, 1H) |
| – | 4.78–4.73 (m, 2H) |
| – | 4.84 (d, *J* = 3.7 Hz, 1H) |
| – | 5.26 (d, *J* = 4.0 Hz, 1H) |
| – | 5.40–5.37 (m, 2H) |
| 7.43 (s, 1H) | 7.49 (s, 1H) |
| 14.3 (s, 1H) | 14.2 (s, 1H) |

Comparison of $^{13}C$ NMR Spectral Data of Natural and Synthetic DC-45-A1 (**10**)

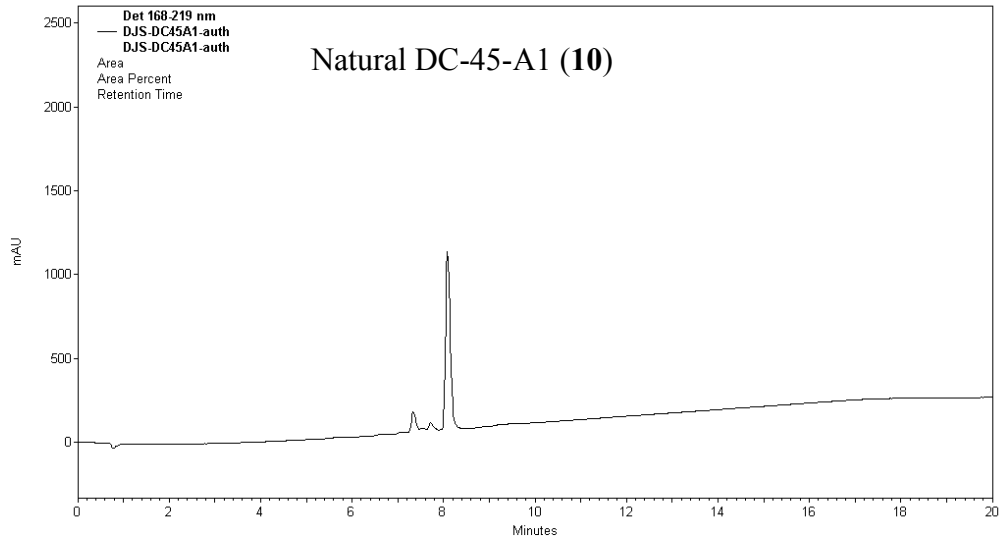
| **Natural**[3] ($CDCl_3$) | **Synthetic** 125 MHz ($CDCl_3$) |
|---|---|
| 16.9 | 16.9 |
| 20.5 | 20.5 |
| 20.9 | 20.9 |
| 25.7 | 25.7 |
| 36.7 | 36.6 |
| 36.7 | 36.7 |
| 50.2 | 50.5 |
| 56.6 | 56.6 |
| 57 | 57.1 |
| 62.7 | 62.7 |
| 63 | 62.9 |
| 68 | 67.7 |
| 68 | 67.9 |
| 68.9 | 68.8 |
| 69.2 | 69.3 |
| 69.4 | 69.5 |
| 73.3 | 73.2 |
| 74.4 | 74.4 |
| 98.3 | 98.1 |
| 99 | 98.6 |
| 100.1 | 100.1 |
| 104.1 | 103.9 |
| 107.1 | 107.3 |
| 114.8 | 114.8 |
| 115.4 | 115.3 |
| 116.7 | 116.8 |
| 126.5 | 126.5 |
| 135.4 | 135.5 |
| 143 | 143 |
| 144.9 | 144.8 |
| 151.6 | 151.7 |
| 163 | 163.1 |
| 170.4 | 170.3 |
| 203.1 | 202.8 |

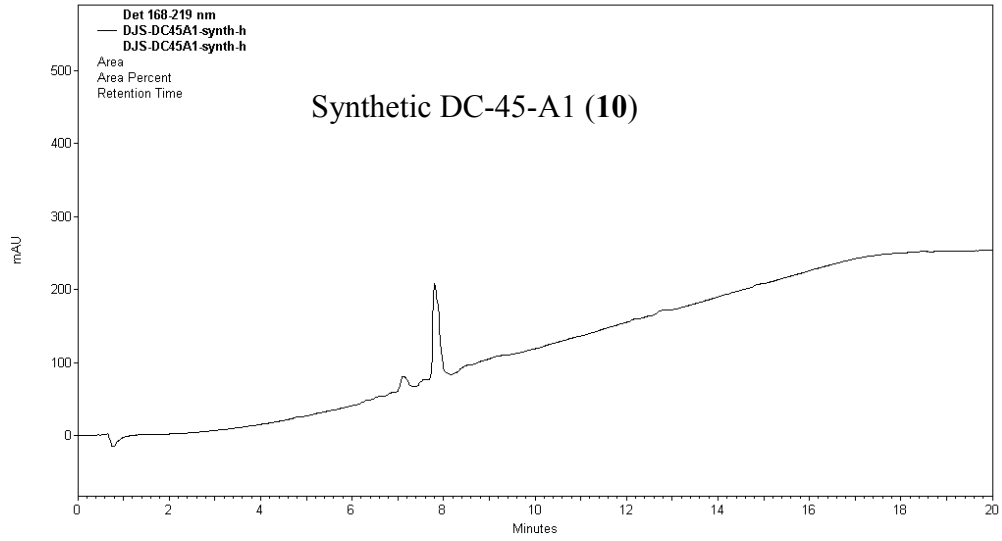
Comparison of Natural and Synthetic DC-45-A1 (**10**) ($^1$H NMR, $CDCl_3$, 500 MHz).

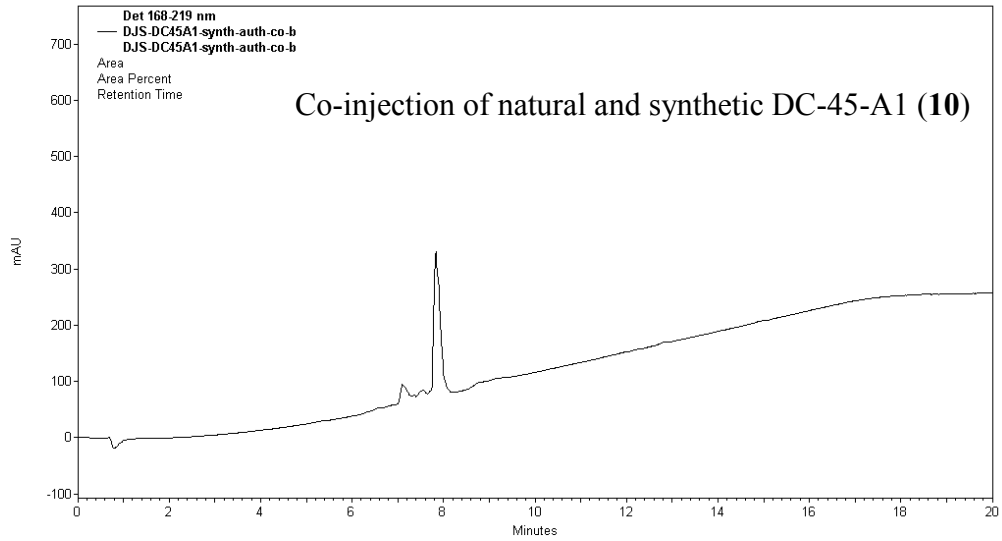
Natural sample obtained from Pfizer, Inc.



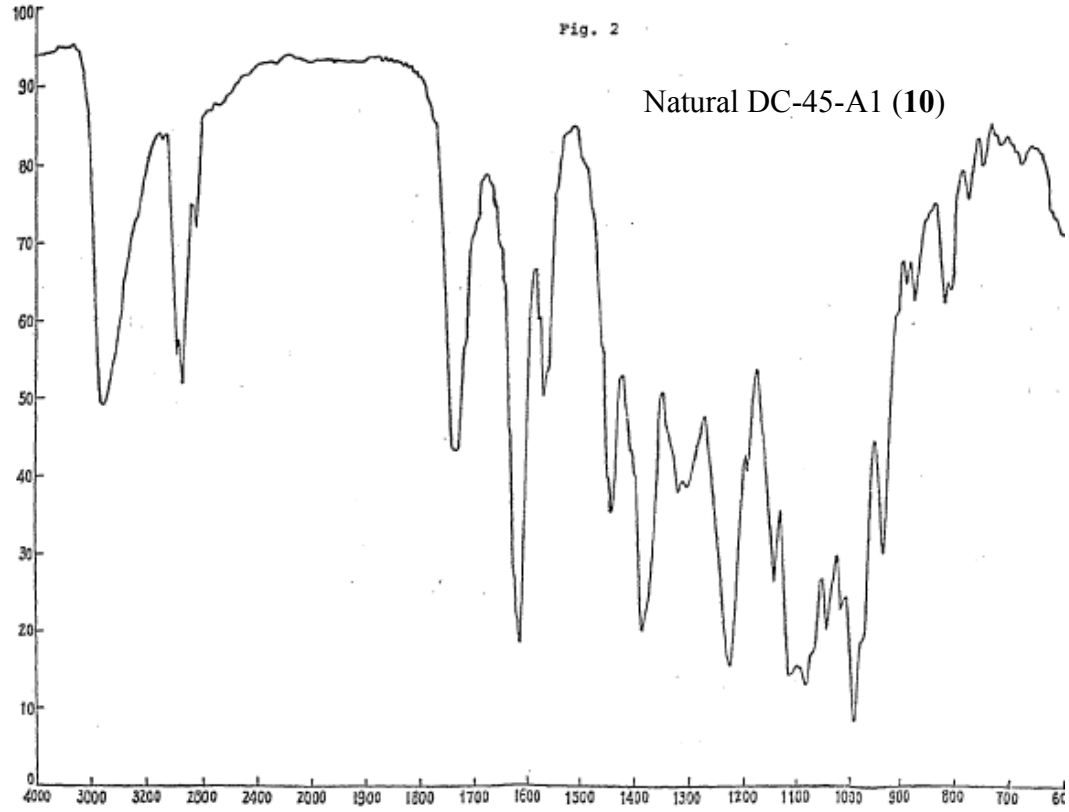
Comparison of Natural and Synthetic DC-45-A1 (**10**)
(RP- HPLC, 10–90% acetonitrile–water containing 0.1% trifluoroacetic acid)

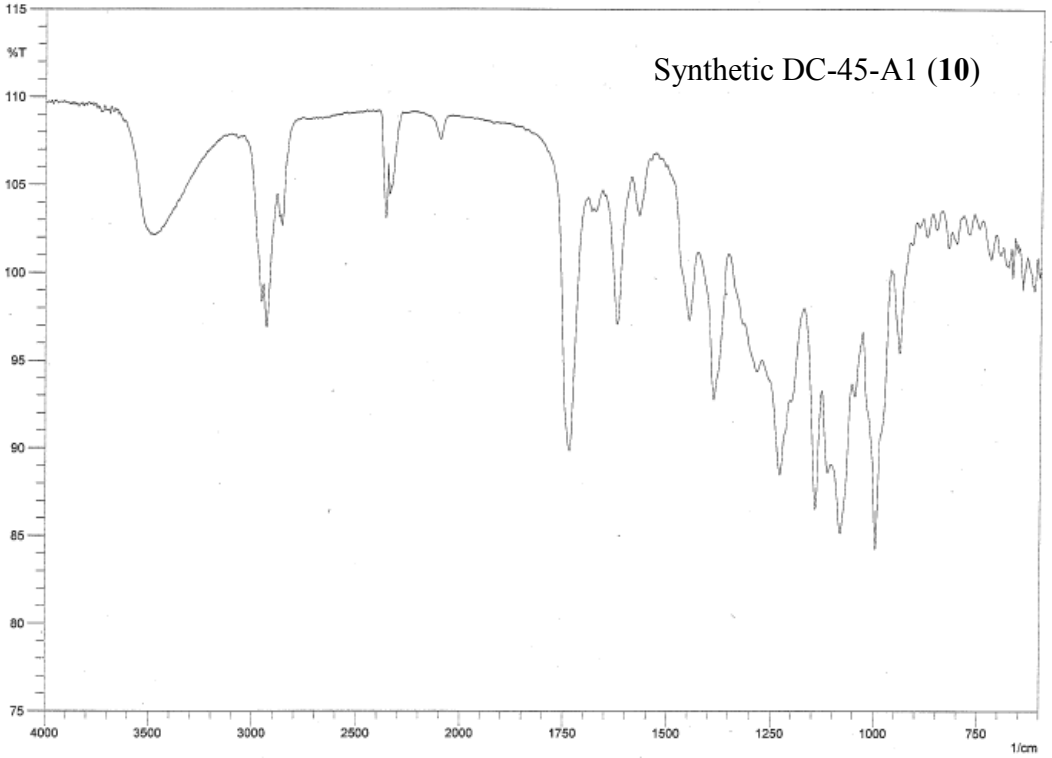


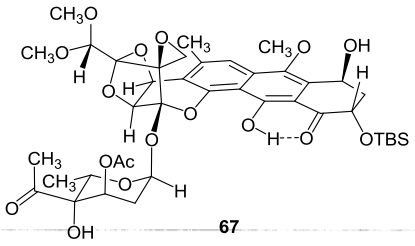



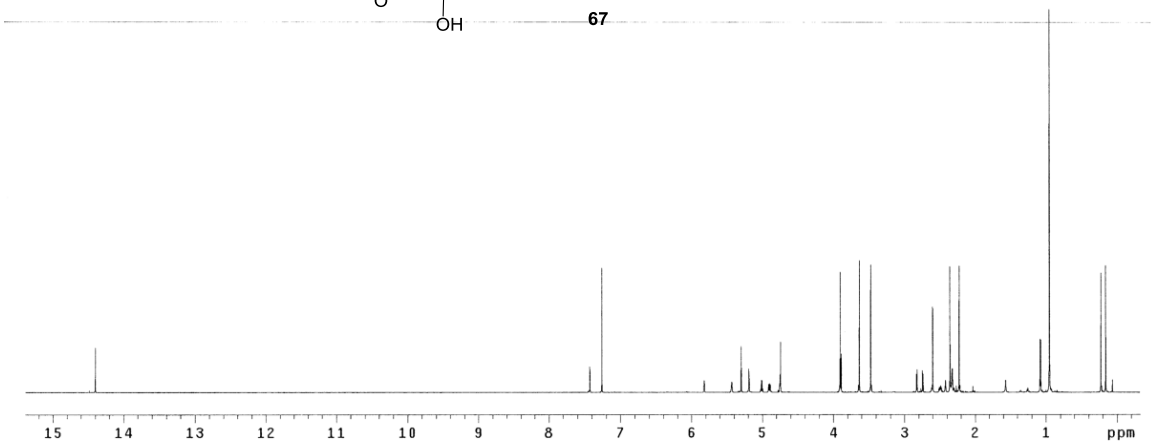
Comparison of Natural[3] and Synthetic DC-45-A1 (**10**) (FT-IR)

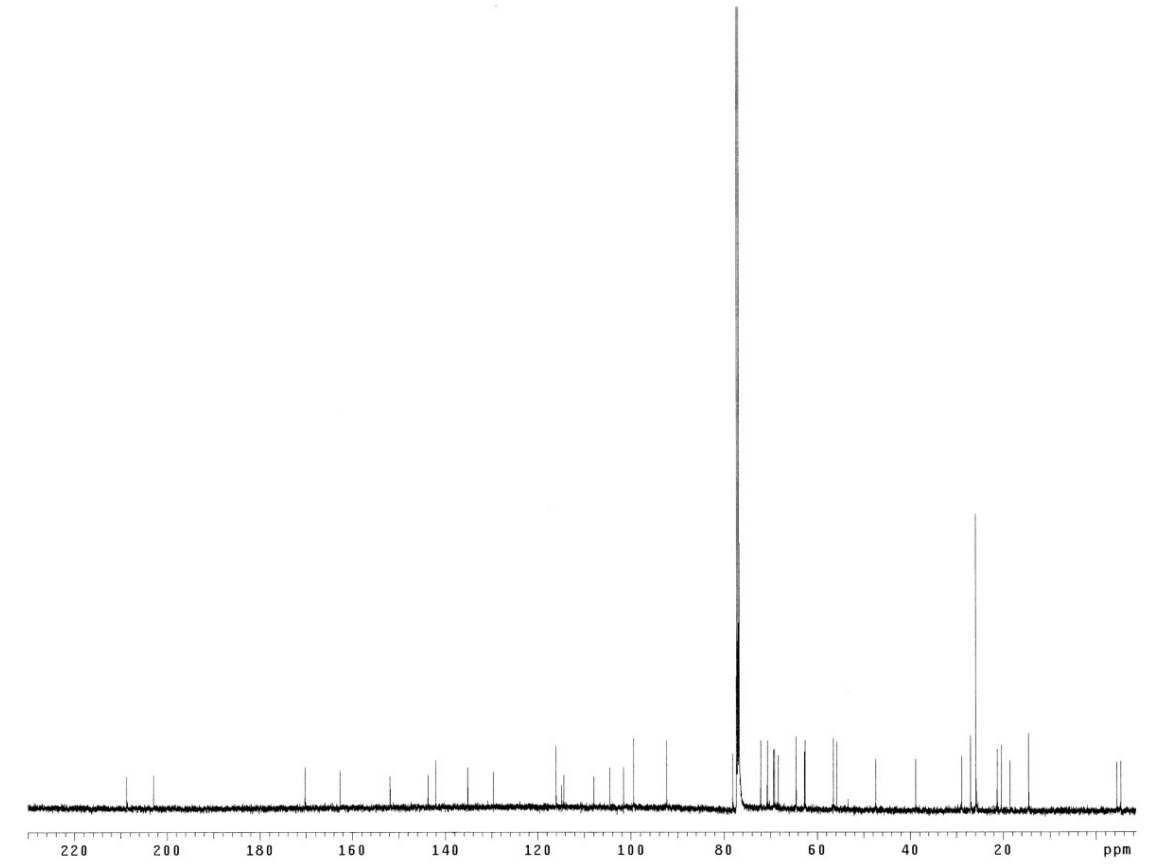


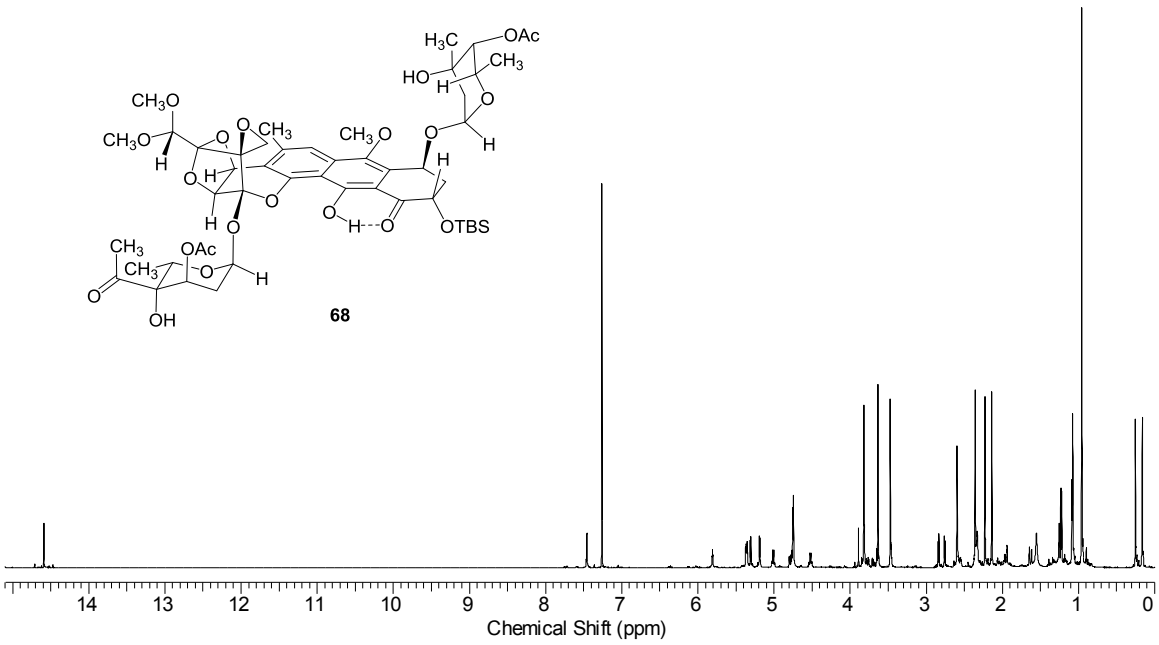



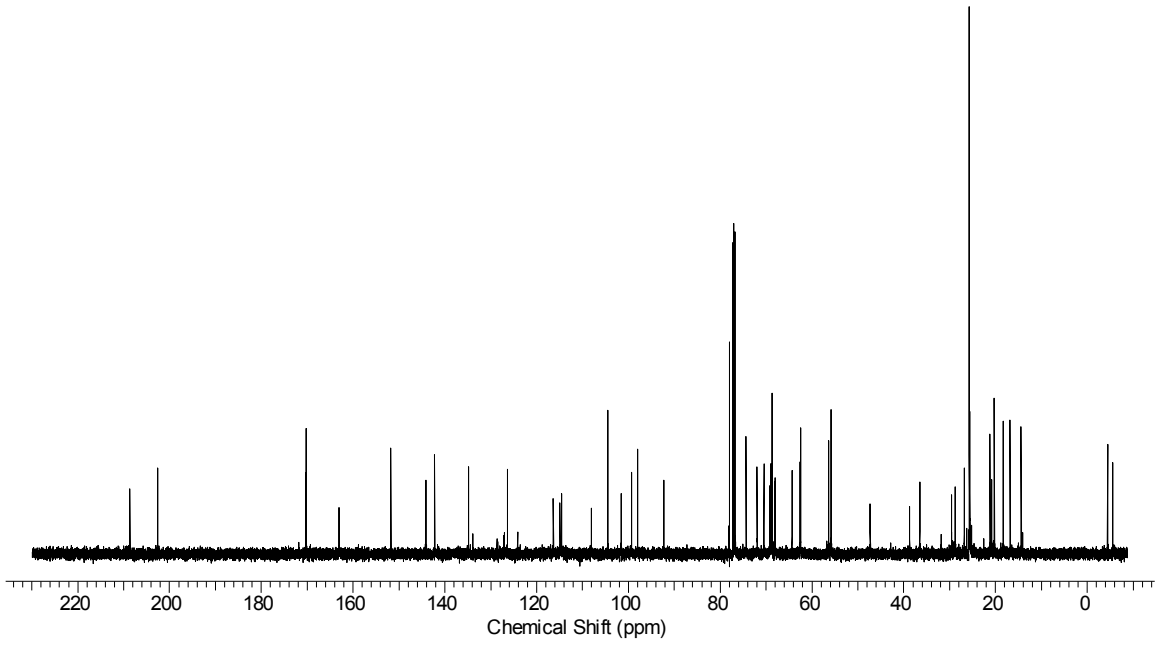

CH3O
CH3O
H
O
O
CH3
CH3O
OH
H
O
H
O
H
O
O
H
O
OTBS
CH3
CH3
OAc
O
O
H
O
OH

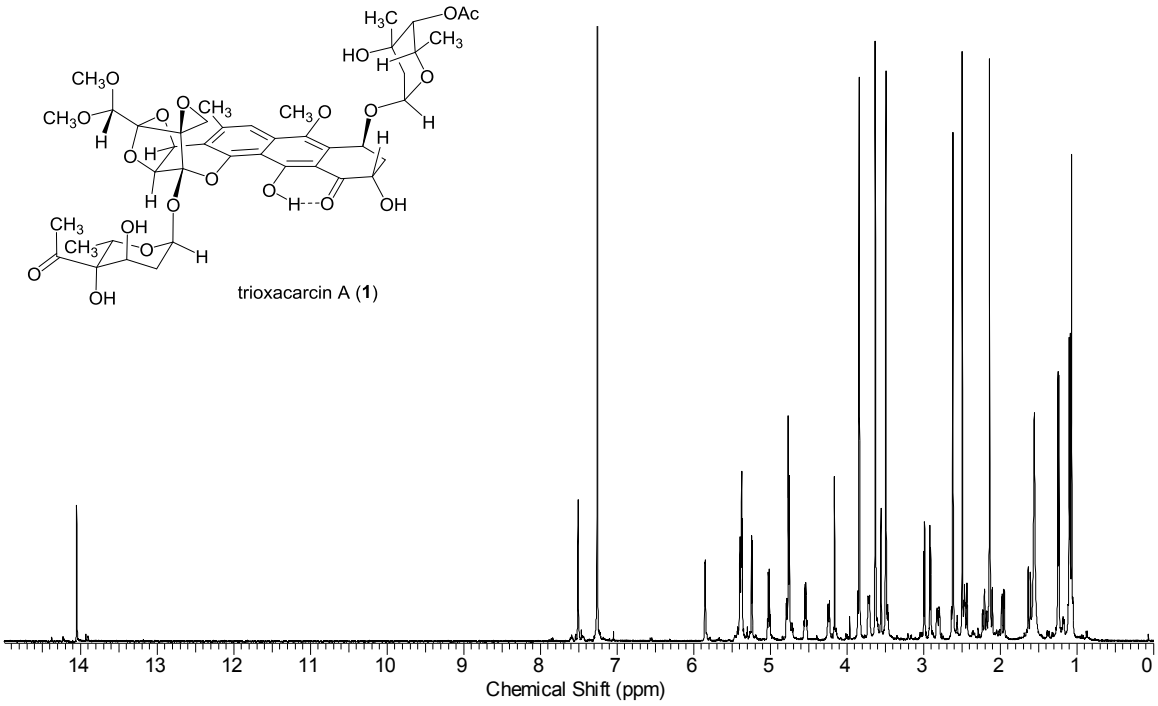
**67**

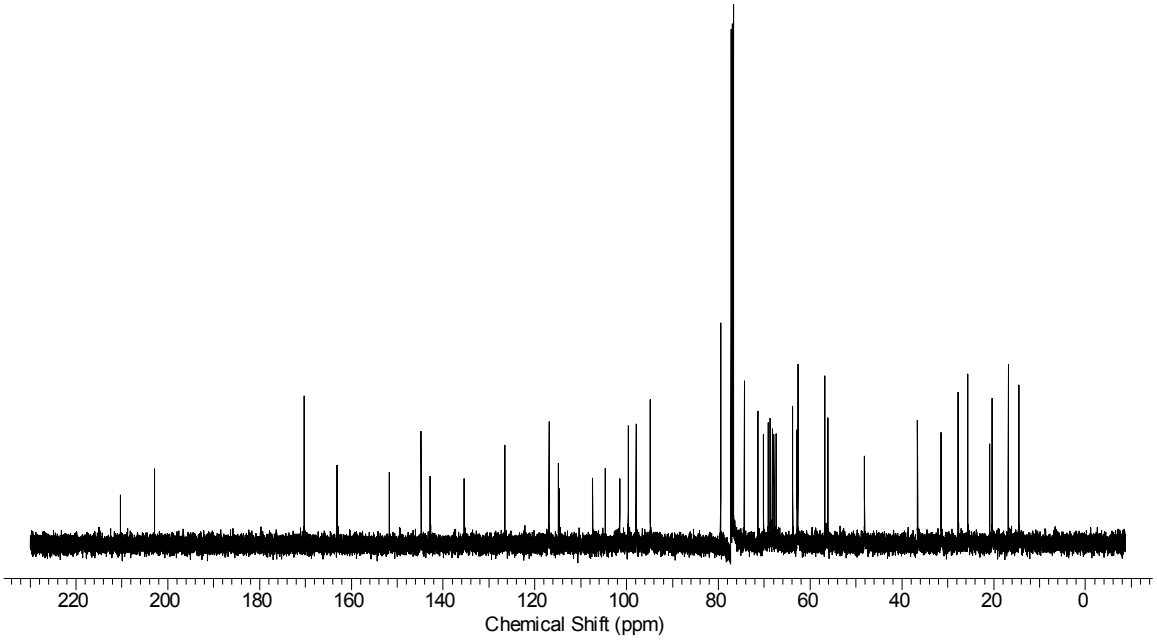

15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm


220
200
180
160
140
120
100
80
60
40
20
ppm

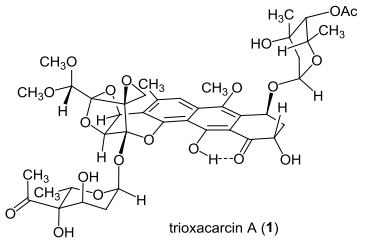

68
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0
Chemical Shift (ppm)


220 200 180 160 140 120 100 80 60 40 20 0
Chemical Shift (ppm)


trioxacarcin A (1)
Chemical Shift (ppm)


Chemical Shift (ppm)

## Comparison of Natural and Synthetic Trioxacarcin A (1)



trioxacarcin A (**1**)

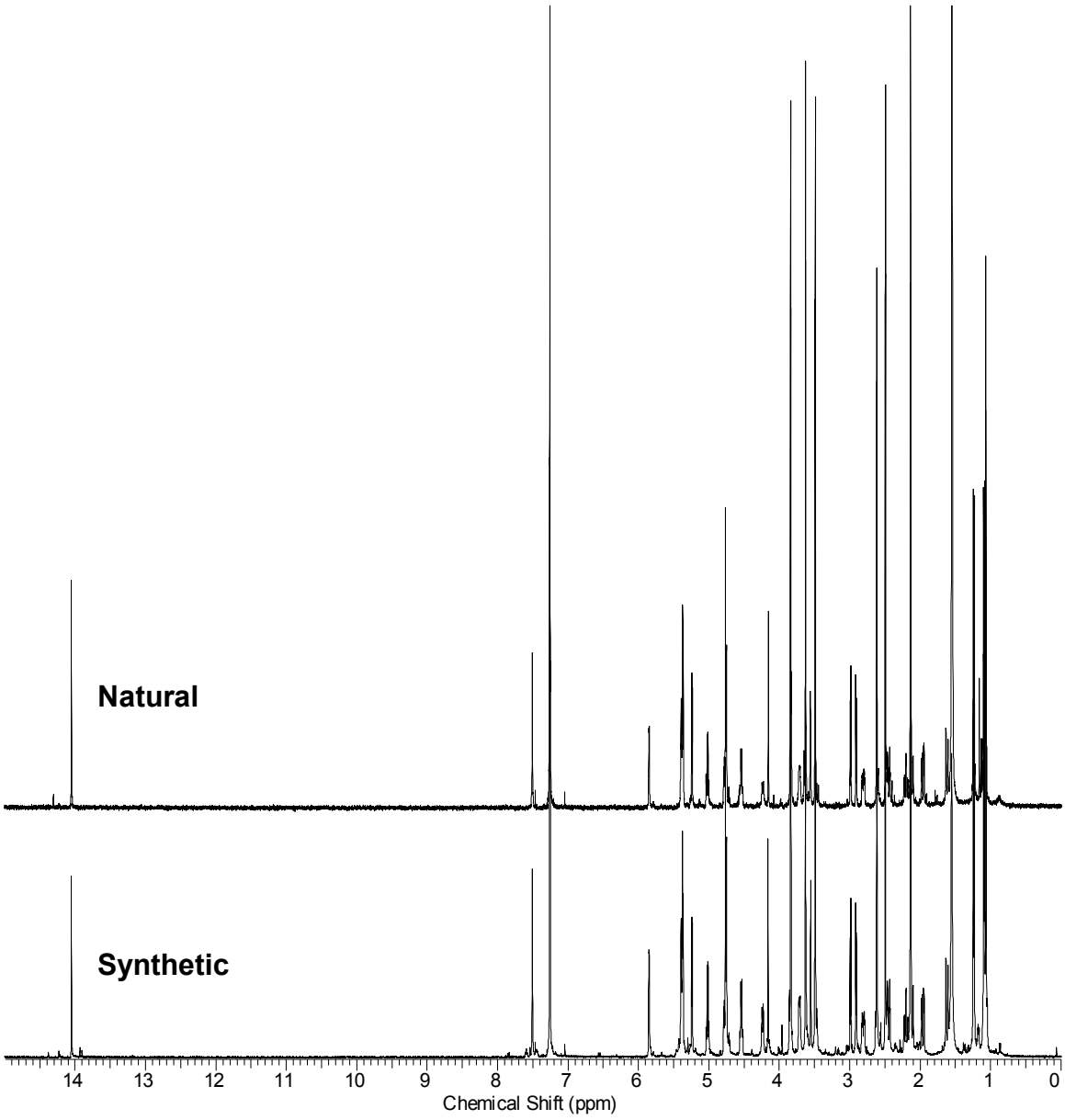
Comparison of $^{1}$H NMR Spectral Data of Natural and Synthetic Trioxacarcin A

| **Natural**[2] | **Natural**[6] | **Synthetic** |
|---|---|---|
| 100 MHz ($CDCl_3$) | 100 MHz ($CDCl_3$) | 500 MHz ($CDCl_3$) |
| 1.07 (s, 3H) | 1.07 (s) | 1.07 (s, 3H) |
| 1.10 (d, *J* = 6.8 Hz, 3H) | 1.10 (d) | 1.10 (d, *J* = 6.2 Hz, 3H) |
| 1.24 (d, *J* = 6.5 Hz, 3H) | 1.24 (d) | 1.24 (d, *J* = 6.6 Hz, 3H) |
| **1.40–2.30** | – | 1.62 (d, *J* = 14.7 Hz, 1H) |
| – | – | 1.97 (dd, *J* = 14.7, 4.0 Hz, 1H) |
| – | – | 2.11 (m, 1H) |
| 2.14 (s, 3H) | 2.14 (s) | 2.14 (s, 3H) |
| – | – | 2.21 (dt, *J* = 13.2, 2.2 Hz, 1H) |
| – | – | 2.45 (dt, *J* = 14.3, 3.3 Hz, 1H) |
| 2.49 (s, 3H) | 2.50 (s) | 2.50 (s, 3H) |
| 2.63 (s, 3H) | 2.62 (s) | 2.62 (s, 3H) |
| **2.30–2.81** | – | 2.81 (m, 1H) |
| 2.91 (d, *J* = 5.6 Hz, 1H) | 2.90 (d) | 2.91 (d, *J* = 5.5 Hz, 1H) |
| 3.00 (d, *J* = 5.6 Hz, 1H) | 3.00 (d) | 2.99 (d, *J* = 5.9 Hz, 1H) |
| 3.49 (s, 3H) | 3.49 (s) | 3.49 (s, 3H) |
| – | – | 3.56 (s, 1H) |
| 3.63 (s, 3H) | 3.63 (s) | 3.63 (s, 3H) |
| **3.60–4.00** | – | 3.72 (br d, *J* = 8.8 Hz, 1H) |
| 3.85 (s, 3H) | 3.85 (s) | 3.84 (s, 3H) |
| 4.18 (s, OH) | 4.16 (s) | 4.16 (s, OH) |
| – | – | 4.24 (d, *J* = 9.2 Hz, 1H) |
| 4.55 (q, *J* = 6.8 Hz, 1H) | 4.55 (q) | 4.54 (q, *J* = 6.6 Hz, 1H) |
| **4.70–4.90** | 4.77 (s) | 4.75 (app s) |
| – | – | 4.77 (s, 1H) |
| – | – | 4.78 (m, 1H) |
| 5.03 (q, *J* = 6.5 Hz, 1H) | 5.02 (q) | 5.02 (q, *J* = 6.6 Hz, 1H) |
| 5.25 (d, *J* = 4.0 Hz, 1H) | 5.24 (d) | 5.24 (d, *J* = 4.0 Hz, 1H) |
| 5.39 (d, *J* = 4.0 Hz, 1H) | 5.38 (d) | 5.37 (d, *J* = 4.0 Hz, 1H) |
| – | – | 5.39 (m, 1H) |
| 5.87 (m, 1H) | 5.87 (d) | 5.85 (d, *J* = 2.6 Hz, 1H) |
| 7.52 (s, 1H) | 7.51 (s) | 7.51 (s, 1H) |
| 14.10 (s, 1H) | 14.1 (s) | 14.05 (s, 1H) |

Comparison of $^{13}C$ NMR Spectral Data of Natural and Synthetic Trioxacarcin A (**1**)

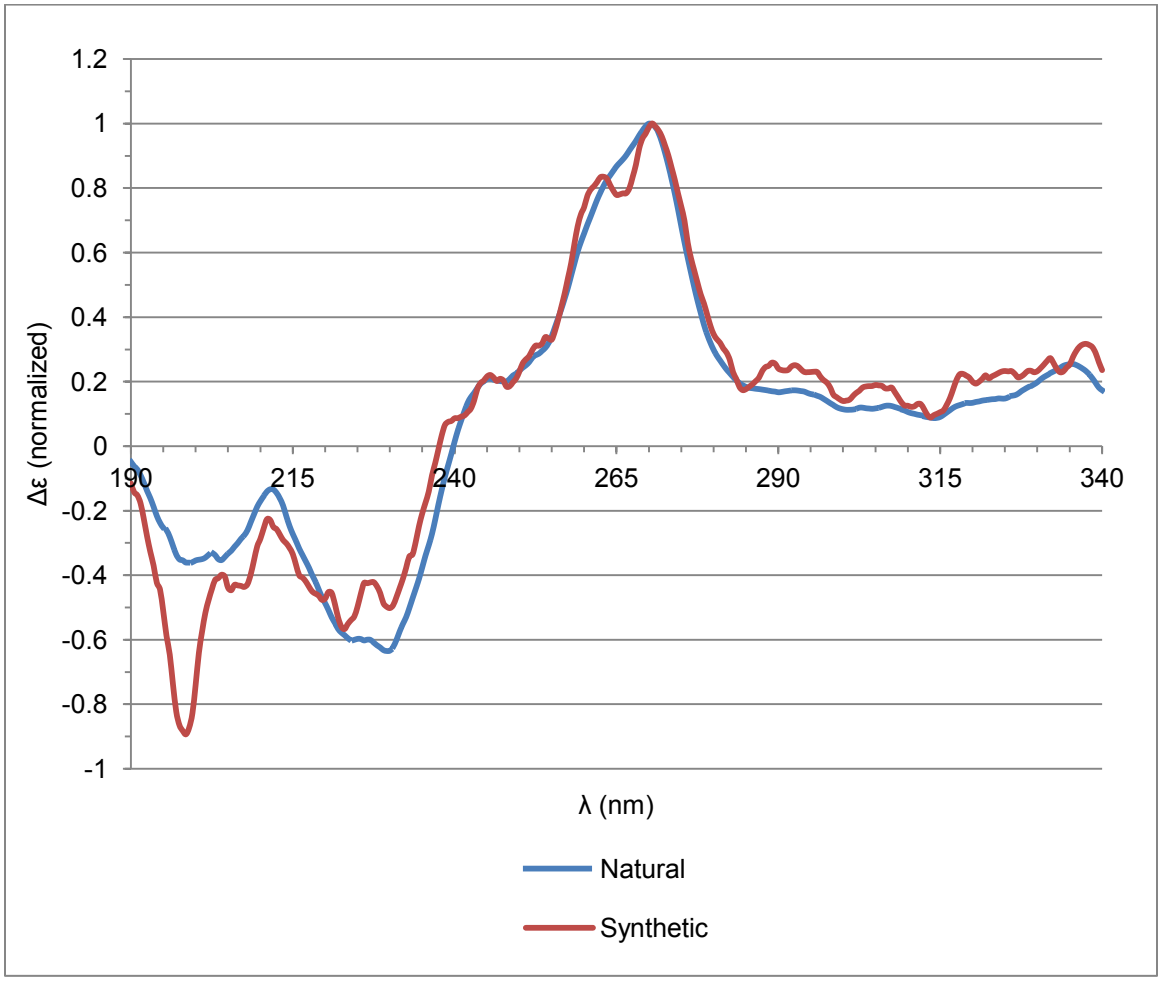
| **Natural**[2] 25 MHz ($CDCl_3$) | **Natural**[6] 25 MHz ($CDCl_3$) | **Synthetic** 125 MHz ($CDCl_3$) |
|---|---|---|
| 14.5 | 14.7 | 14.5 |
| 16.8 | 17.0 | 16.8 |
| 20.3 | 20.2 | 20.3 |
| 20.8 | 20.9 | 20.9 |
| 25.7 | 25.7 | 25.7 |
| 27.7 | 28.0 | 27.8 |
| 31.5 | 32.2 | 31.5 |
| 36.7 (2 × $CH_2$) | 36.5 | 36.6 |
| 48.1 | – | 48.1 |
| 56.2 | 55.9 | 56.2 |
| 56.7 | 57.3 | 56.8 |
| 62.7 | – | 62.7 |
| 62.9 | 62.8 | 62.9 |
| 63.8 | 64.0 | 63.8 |
| 67.6 | 66.3 | 67.4 |
| 67.9 | 67.9 | 67.9 |
| 68.3 | 68.8 | 68.3 |
| 68.8 | 69.3 | 68.8 |
| 69.1 | 69.6 | 69.1 |
| 70.2 | – | 70.2 |
| 71.5 | 71.1 | 71.4 |
| 74.4 | 74.6 | 74.4 |
| 79.5 | 79.0 | 79.5 |
| 94.8 | 85.1 | 94.8 |
| 98.0 | 93.7 | 97.9 |
| 99.7 | 97.2 | 99.7 |
| 101.5 | 99.7 | 101.5 |
| 104.6 | 105.3 | 104.6 |
| 107.5 | 108.3 | 107.4 |
| 114.8 (2 × $C_q$) | 114.2 | 114.8 |
| 116.9 | 117.0 | 116.9 |
| 126.6 | 126.7 | 126.6 |
| 135.4 | 135.3 | 135.4 |
| 142.8 | 142.3 | 142.8 |
| 144.7 | 145.2 | 144.8 |
| 151.7 | 152.5 | 151.7 |
| 163.1 | 162.1 | 163.1 |
| 170.2 | 170.3 | 170.3 |
| 203.0 | 203.8 | 202.9 |
| 210.3 | 210.9 | 210.3 |

Comparison of Natural and Synthetic Trioxacarcin A (**1**) ($^1$H NMR, $CDCl_3$, 500 MHz). Natural sample obtained from Pfizer, Inc.

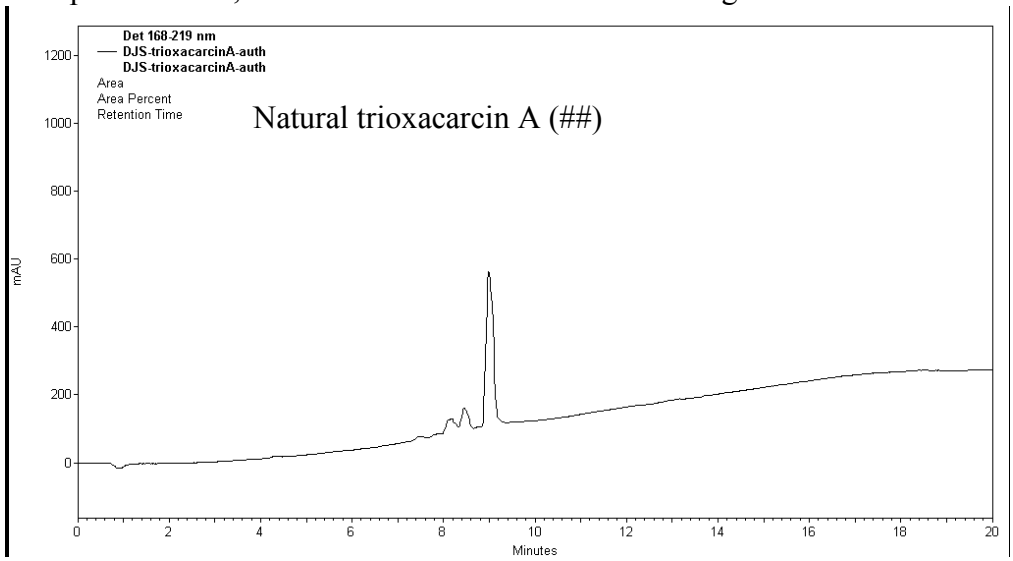


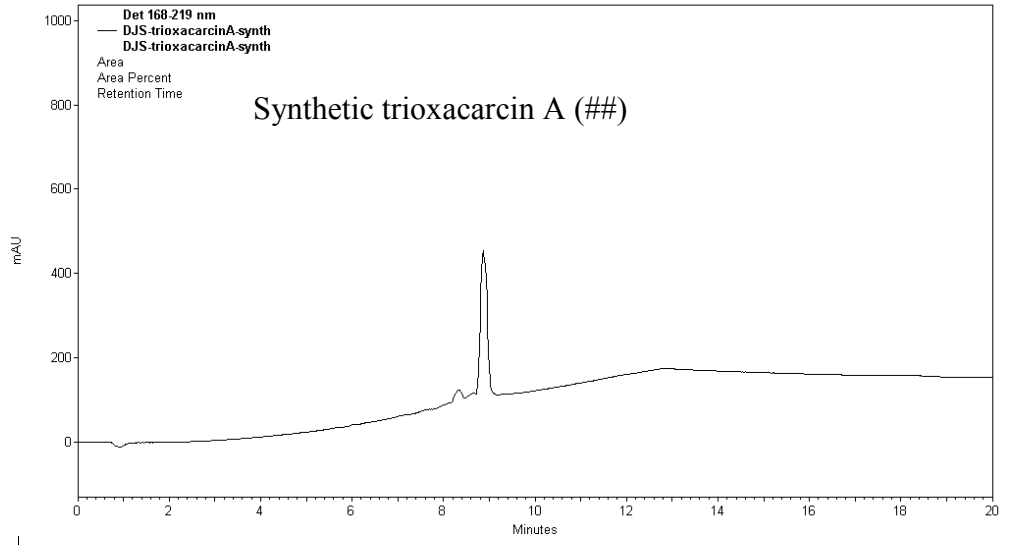
Comparison of Natural and Synthetic Trioxacarcin A (**1**) (Circular Dichroism).

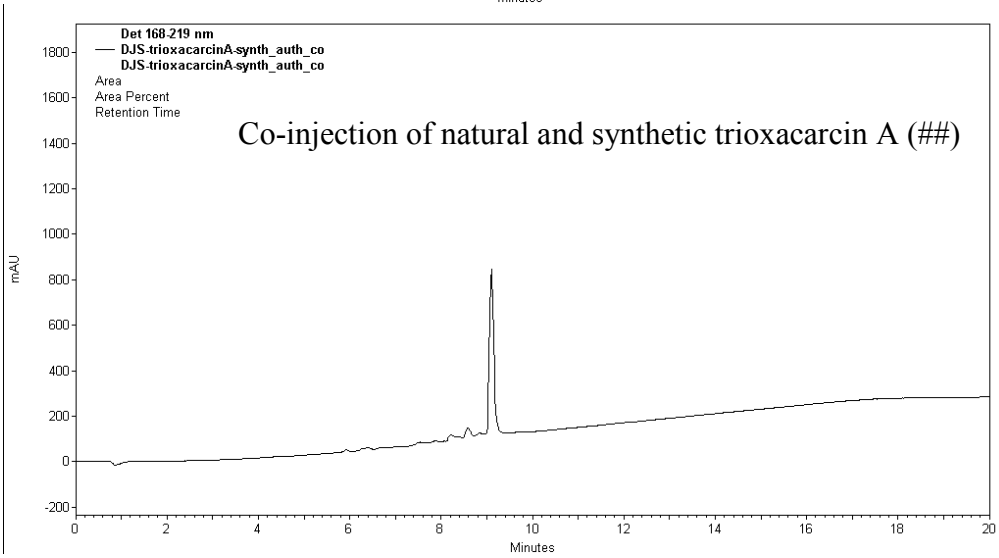
Natural sample obtained from Pfizer, Inc.
Ellipticity (Δε) values are normalized such that the maximum for each sample (at 270 nm) equals 1.



Comparison of Natural and Synthetic Trioxacarcin A (**1**)
(reversed-phase HPLC, 10–90% acetonitrile–water containing 0.1% trifluoroacetic acid)

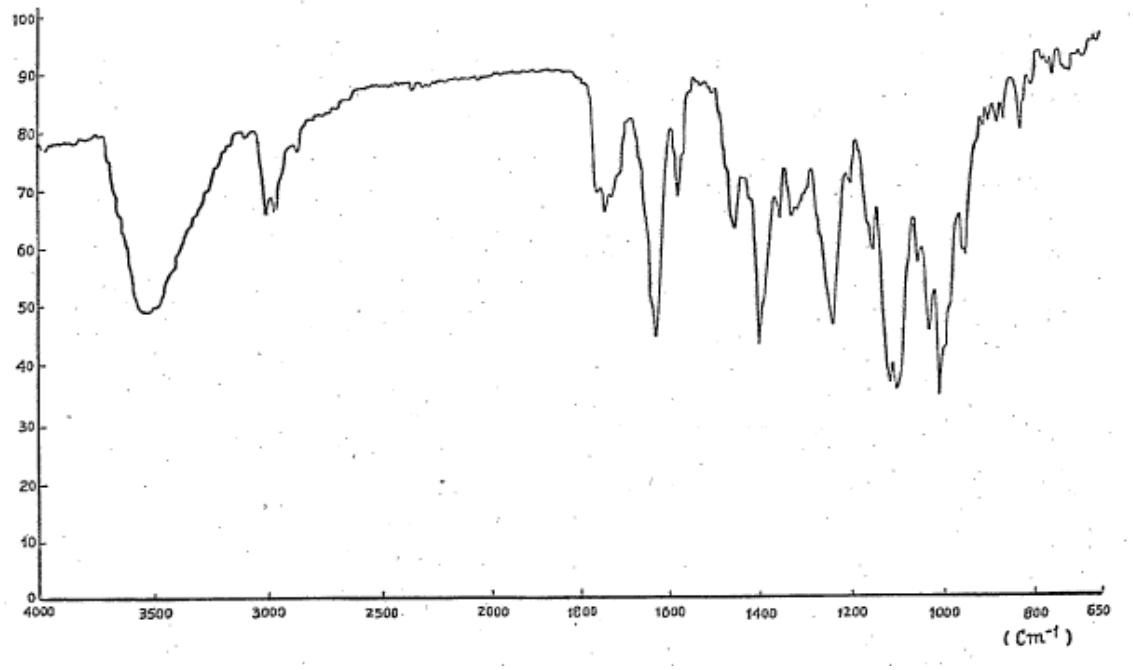


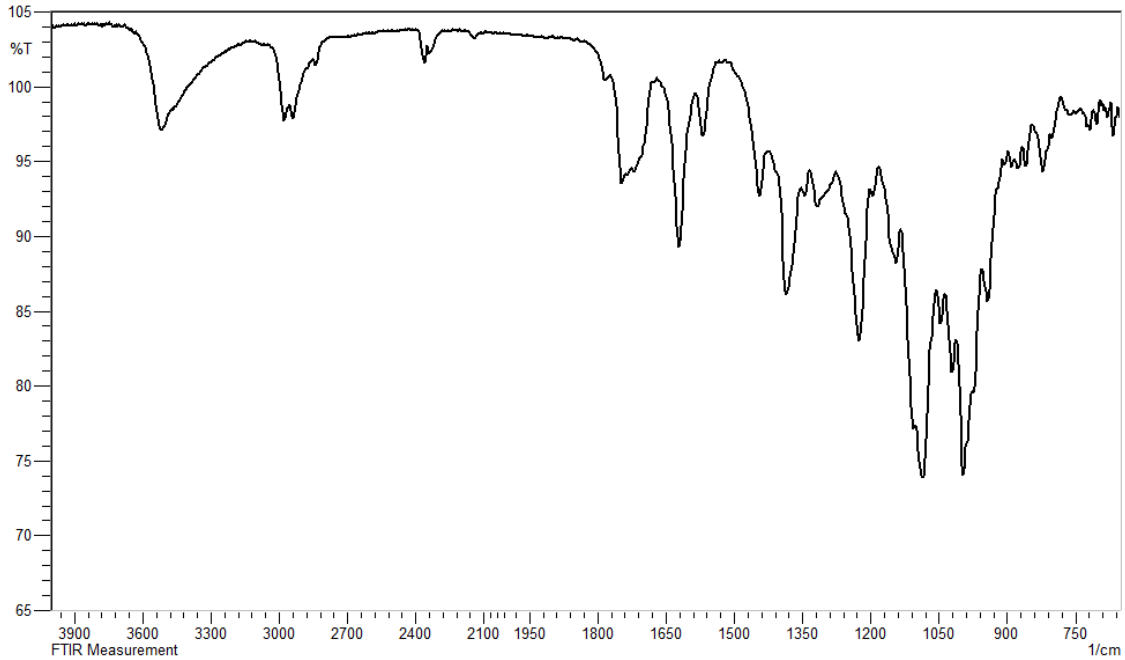



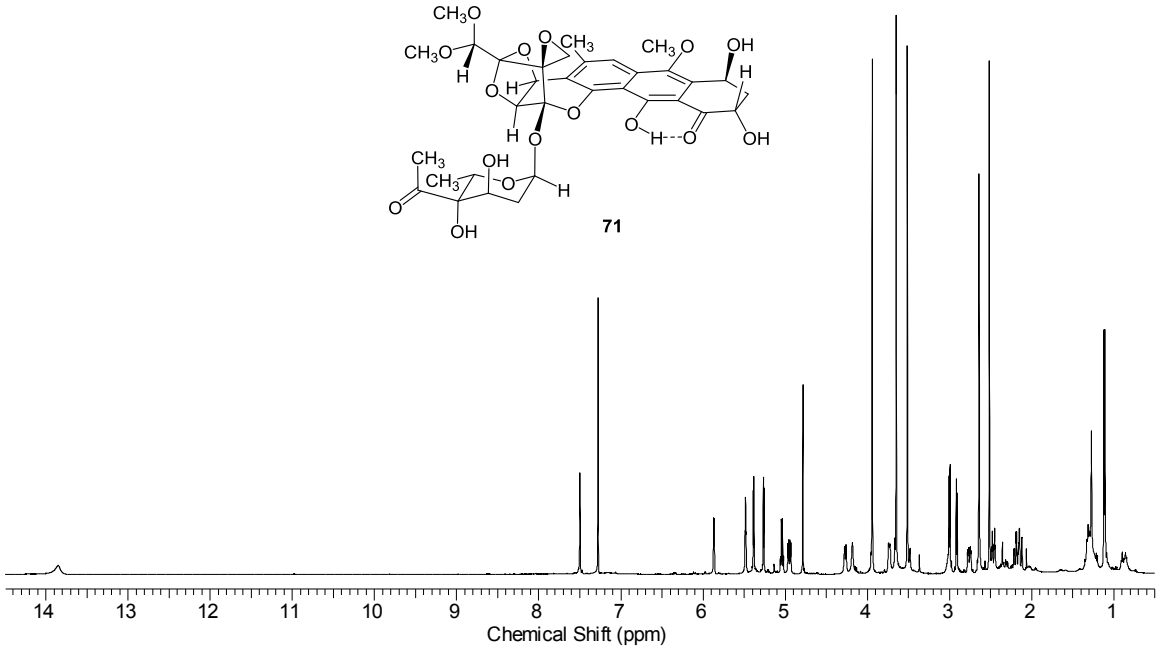
Comparison of Natural[2] and Synthetic Trioxacarcin A (**1**) (FT-IR)

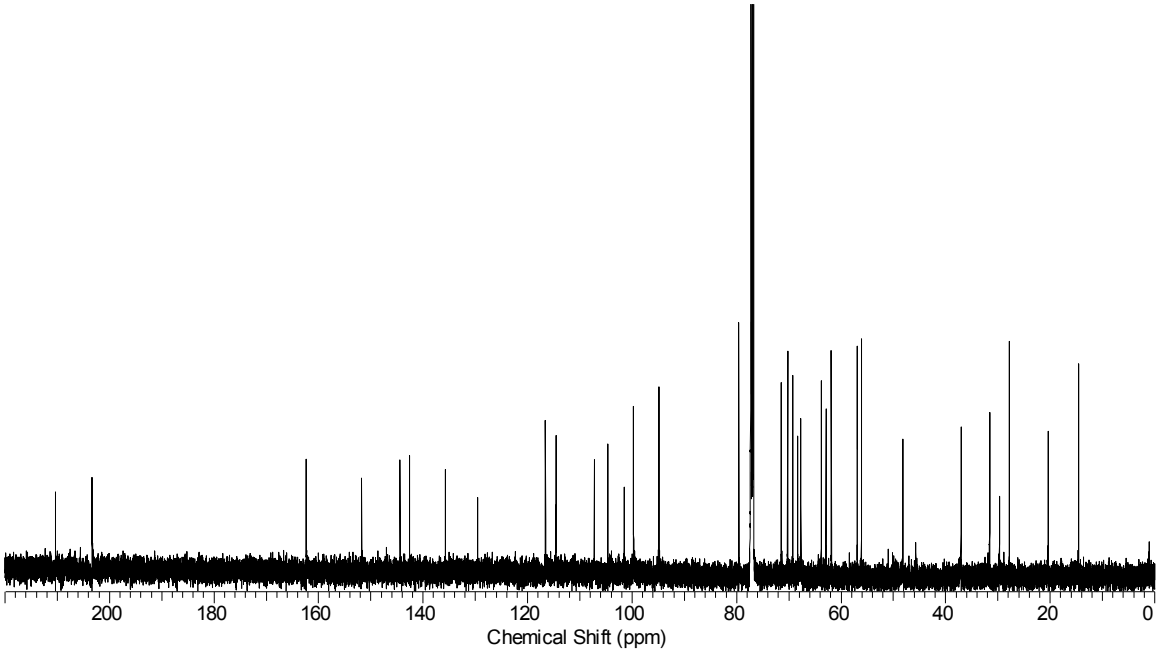
Natural Trioxacarcin A (**1**)

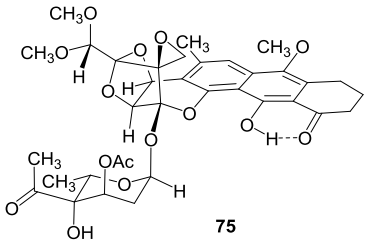



Synthetic Trioxacarcin A (**1**)

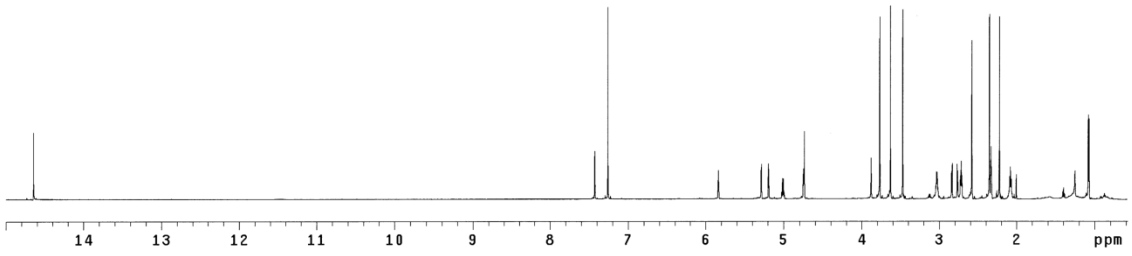


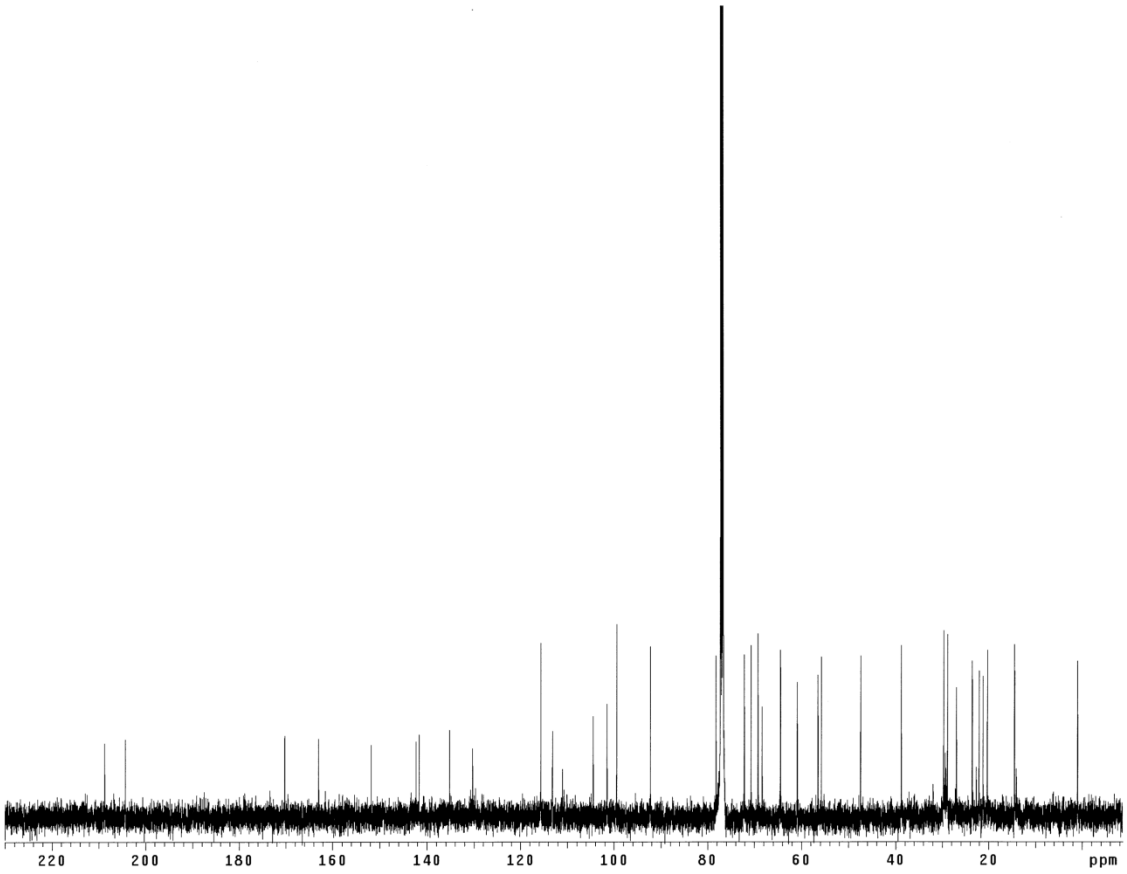

71
14 13 12 11 10 9 8 7 6 5 4 3 2 1
Chemical Shift (ppm)

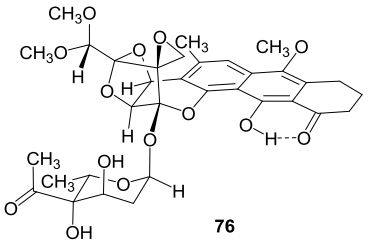

200 180 160 140 120 100 80 60 40 20 0
Chemical Shift (ppm)


CH3O
CH3O
H
O
CH3
CH3O
O
H
O
H
O
O
H
O
CH3
CH3
OAc
O
O
H
OH

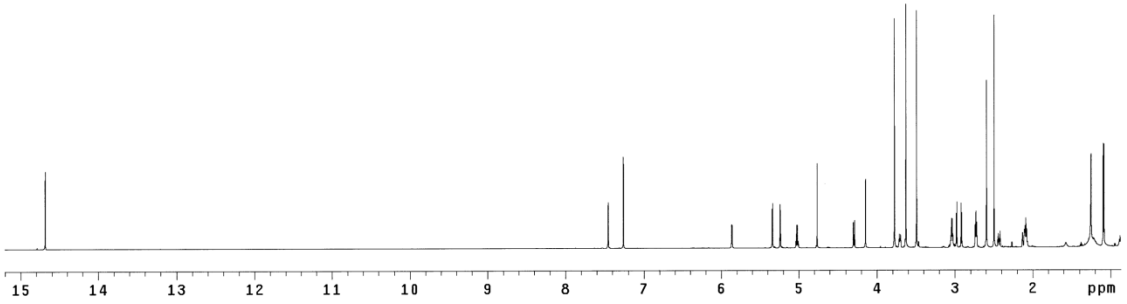
**75**

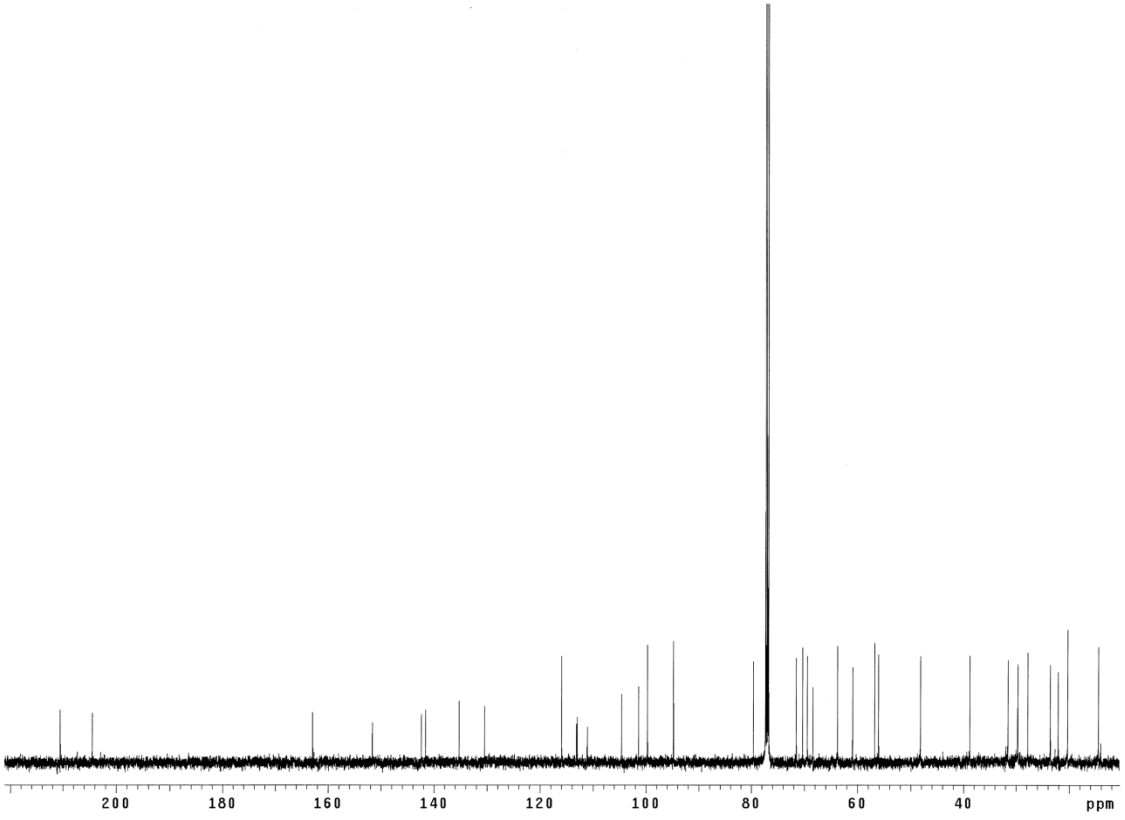

14
13
12
11
10
9
8
7
6
5
4
3
2
ppm

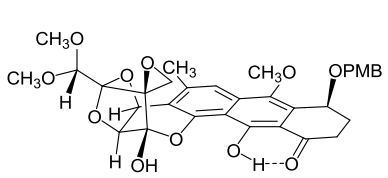

220
200
180
160
140
120
100
80
60
40
20
ppm



**76**

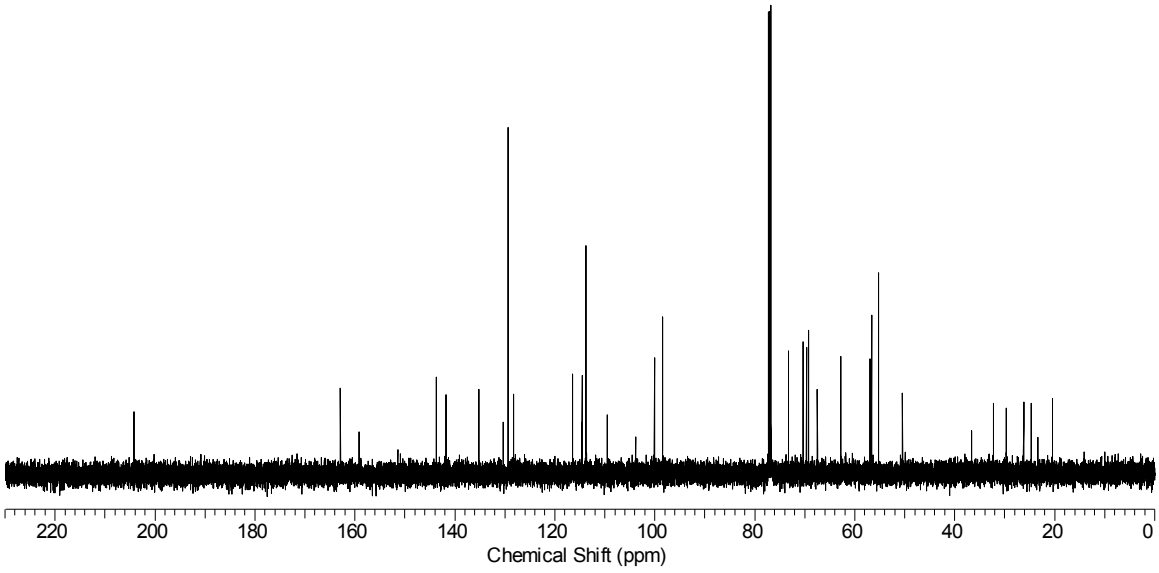

15
14
13
12
11
10
9
8
7
6
5
4
3
2
ppm

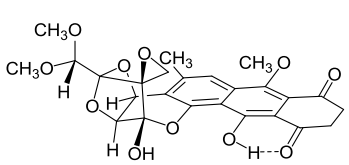

200
180
160
140
120
100
80
60
40
ppm


CH3O
CH3O
H
O
O
CH3
CH3O
OPMB
O
H
H
O
H
OH
O
H---O

**78**

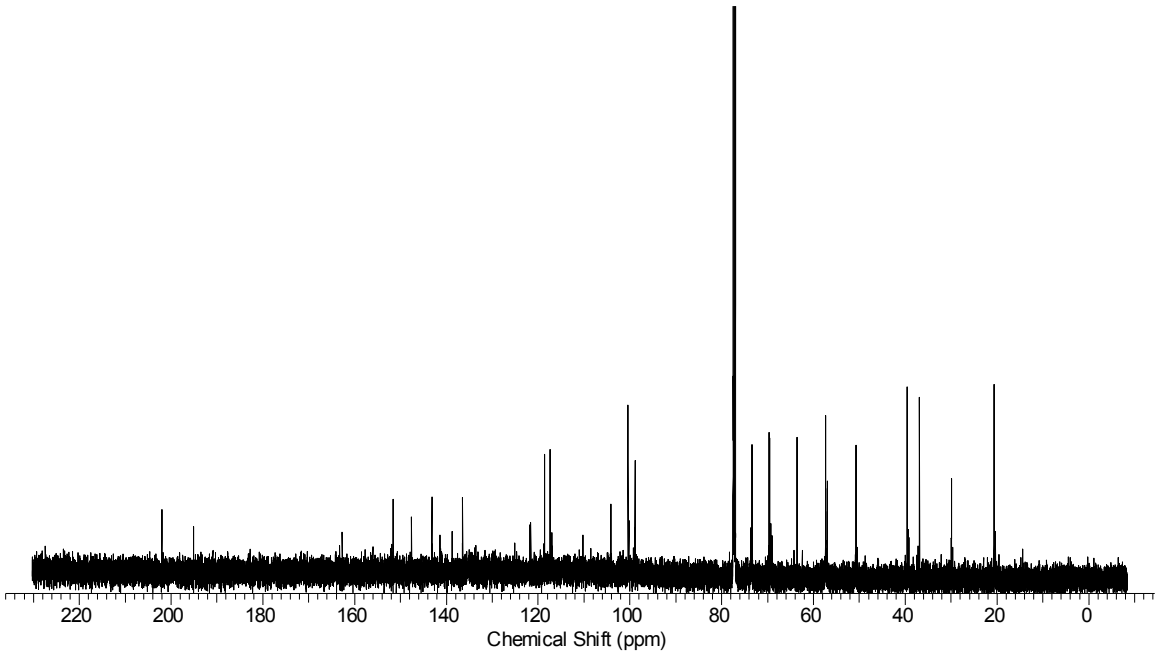

15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

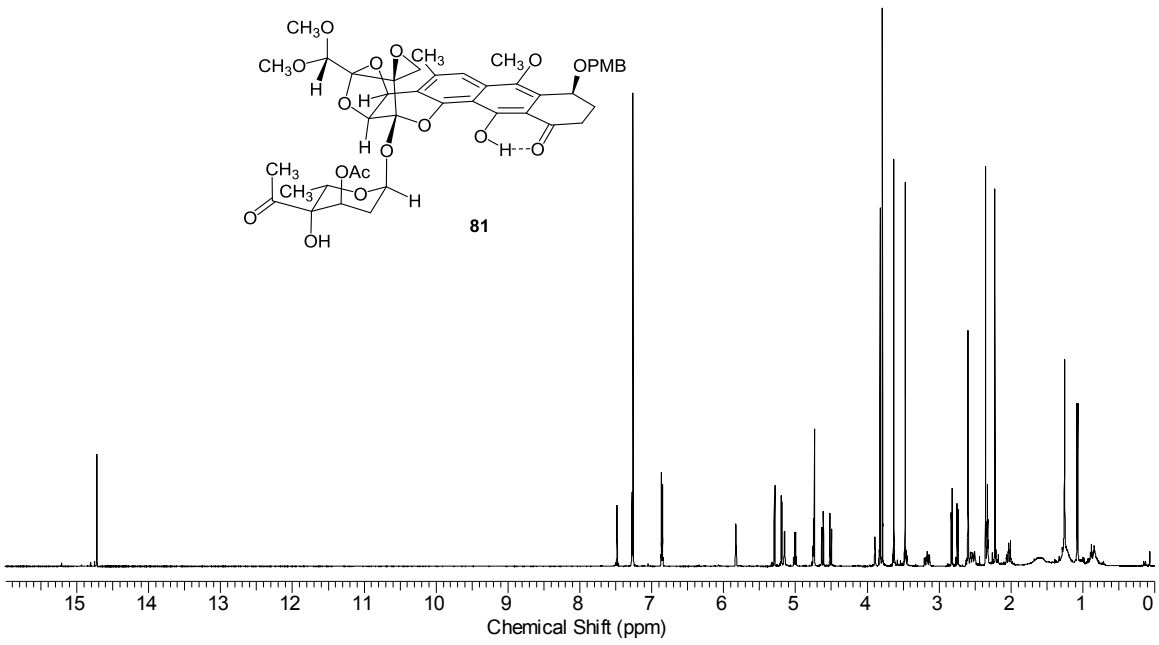

220
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)



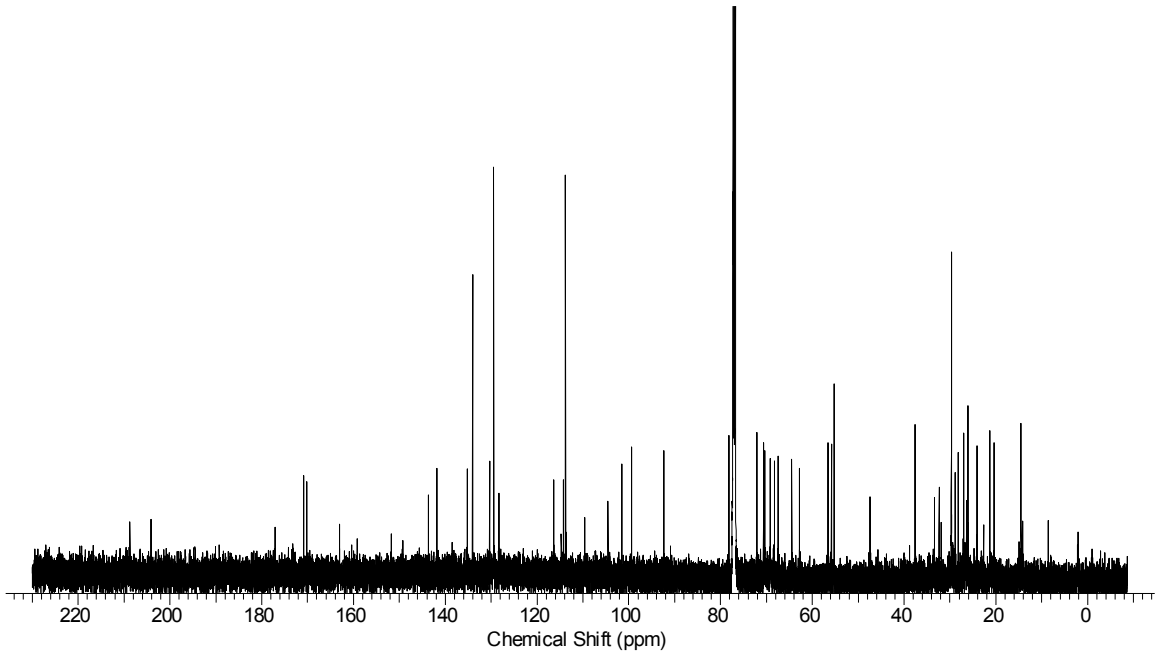
**82**

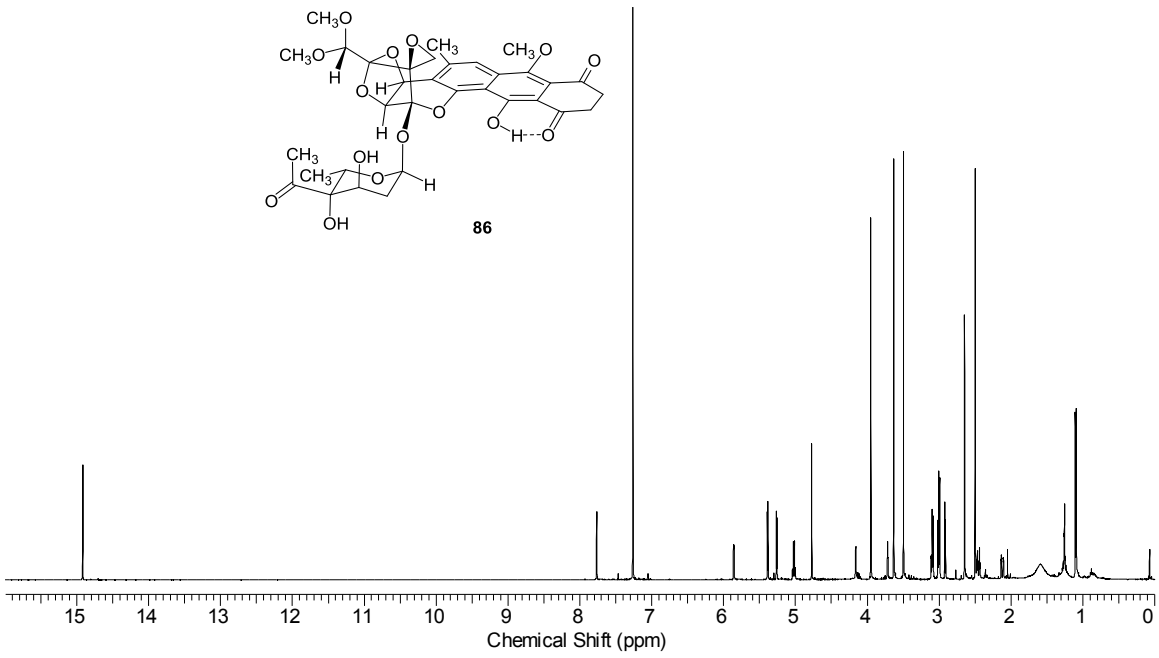

15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0
Chemical Shift (ppm)


220 200 180 160 140 120 100 80 60 40 20 0
Chemical Shift (ppm)

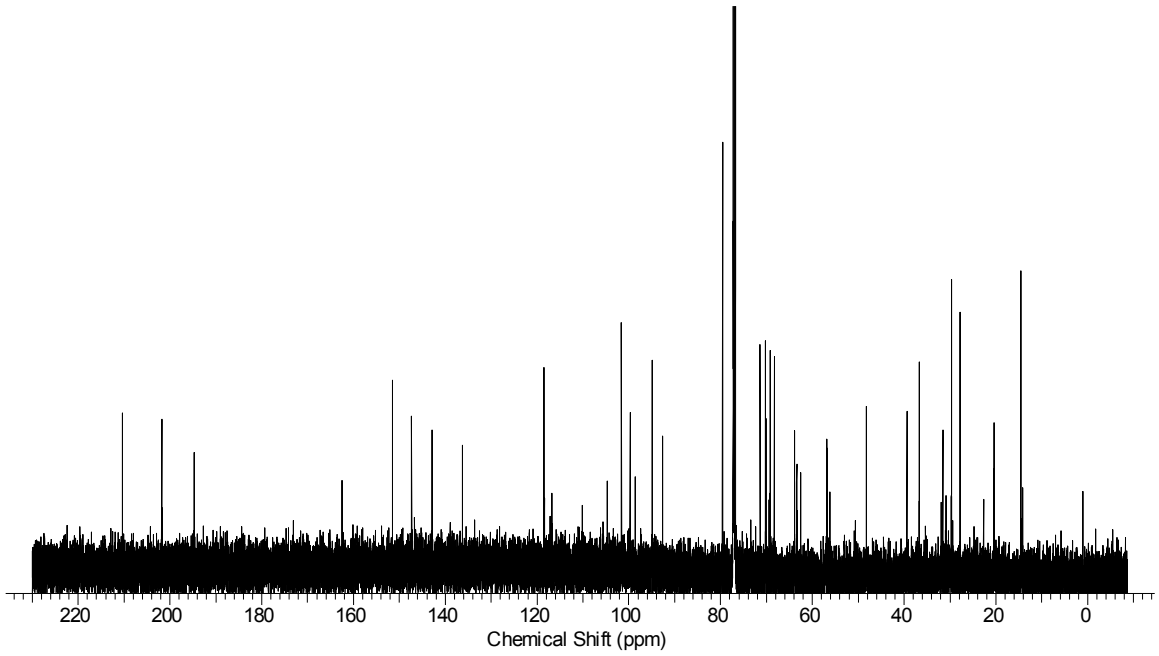

15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0
Chemical Shift (ppm)

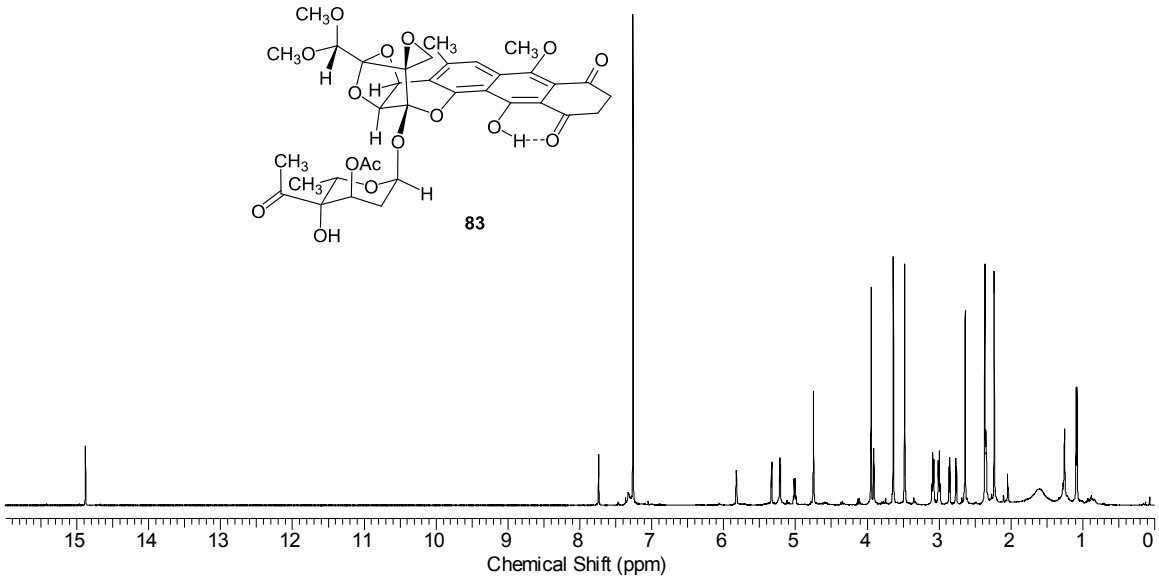
81


220 200 180 160 140 120 100 80 60 40 20 0
Chemical Shift (ppm)

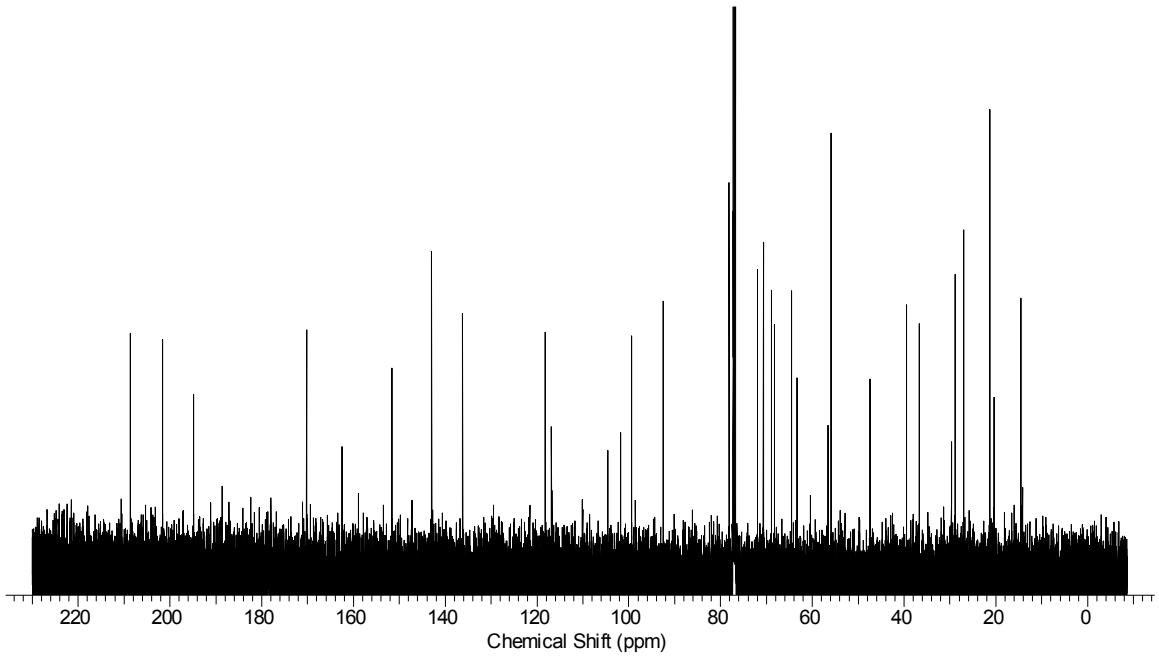

15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0
Chemical Shift (ppm)

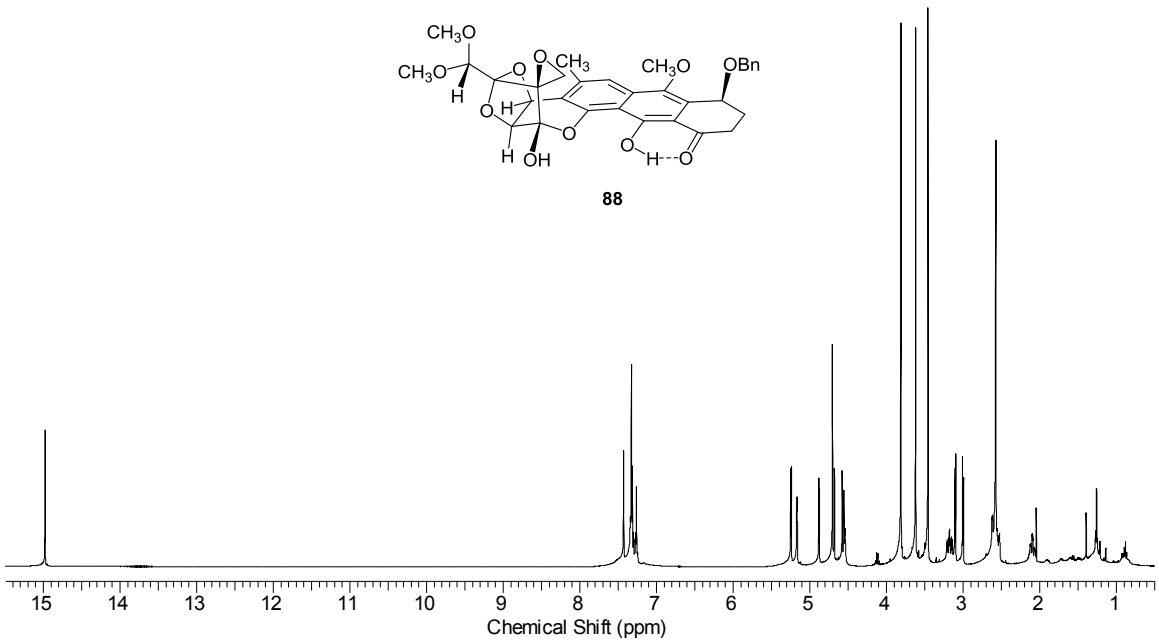
**86**


220 200 180 160 140 120 100 80 60 40 20 0
Chemical Shift (ppm)

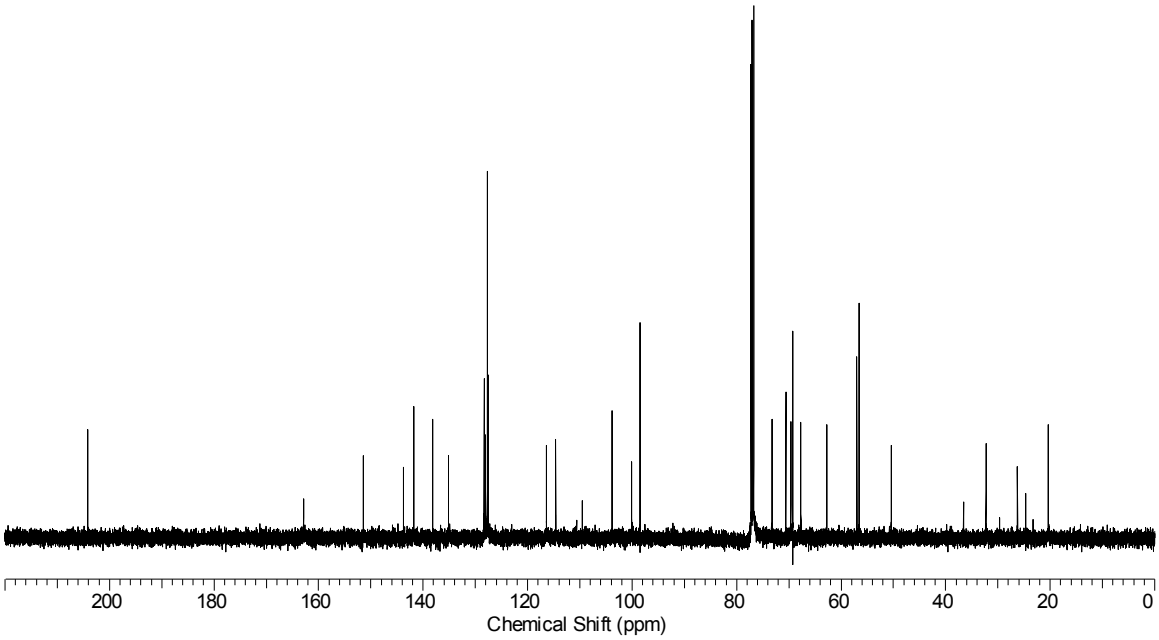

15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0
Chemical Shift (ppm)

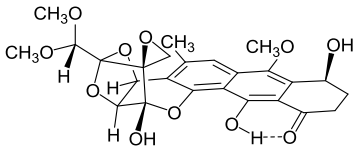
**83**


220 200 180 160 140 120 100 80 60 40 20 0
Chemical Shift (ppm)


15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
Chemical Shift (ppm)

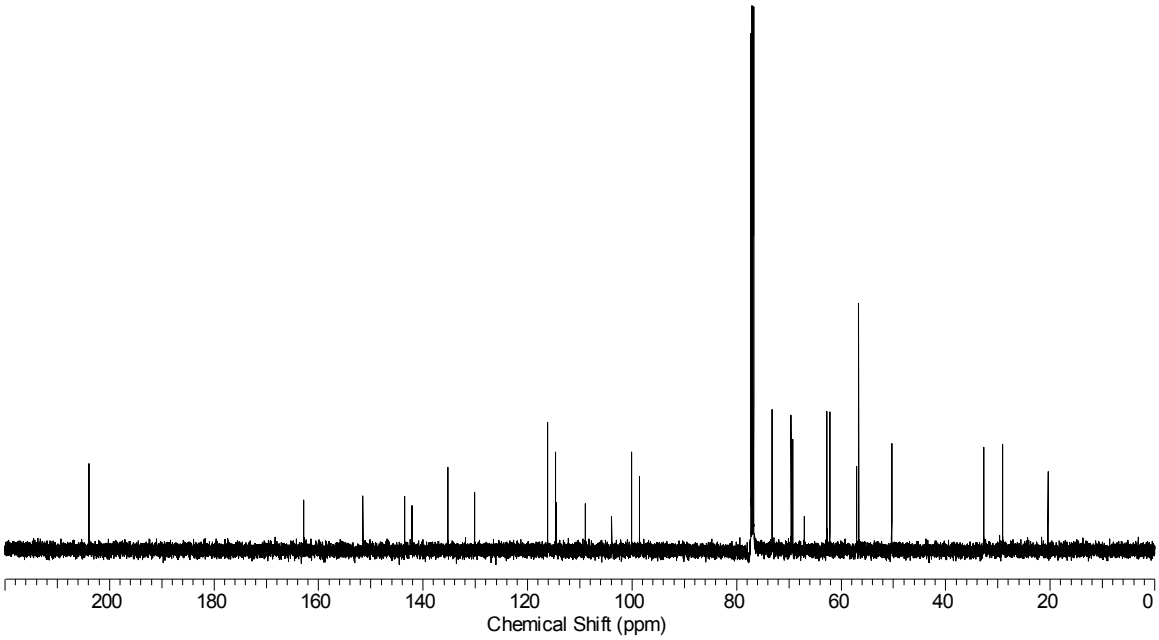
**88**

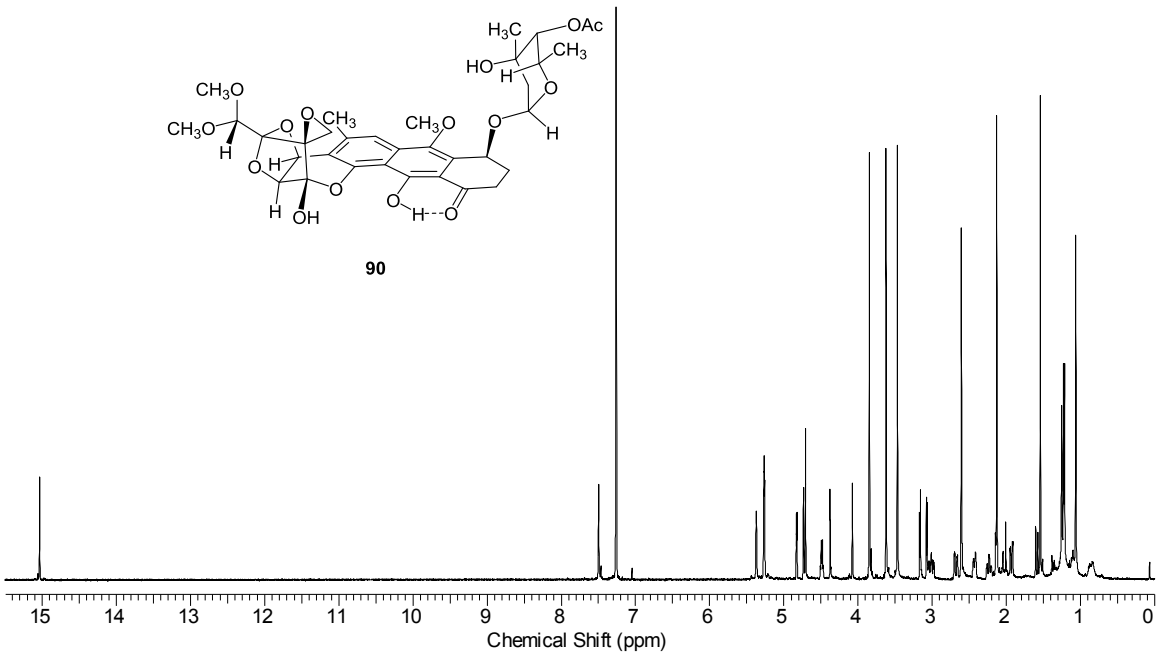

200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)



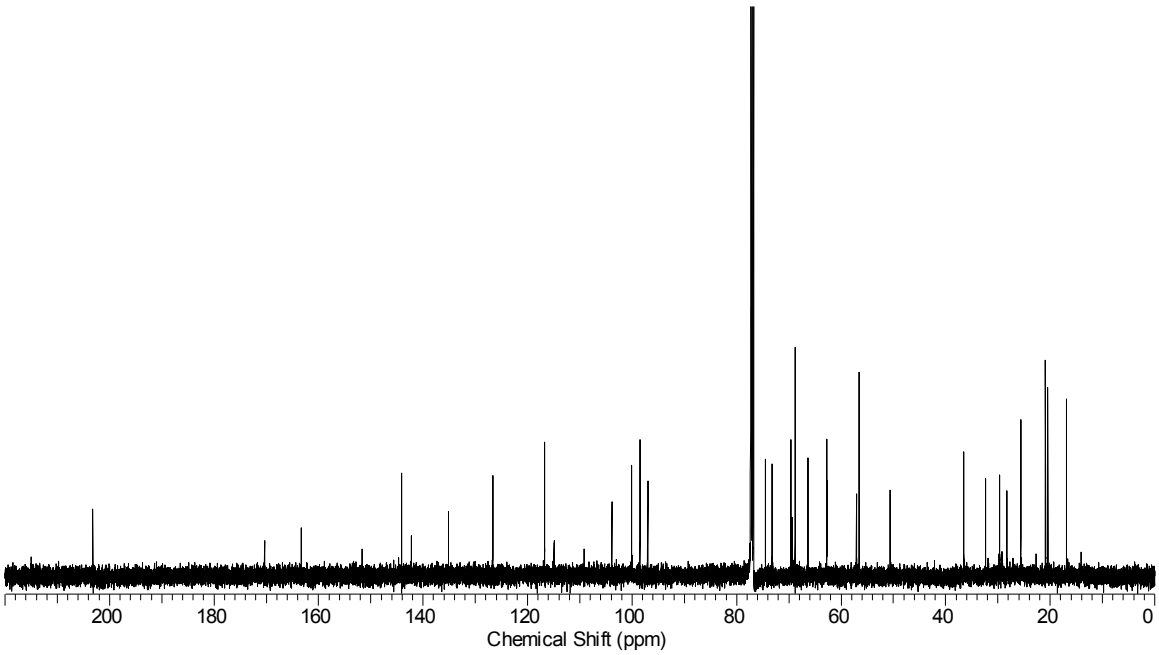
**89**

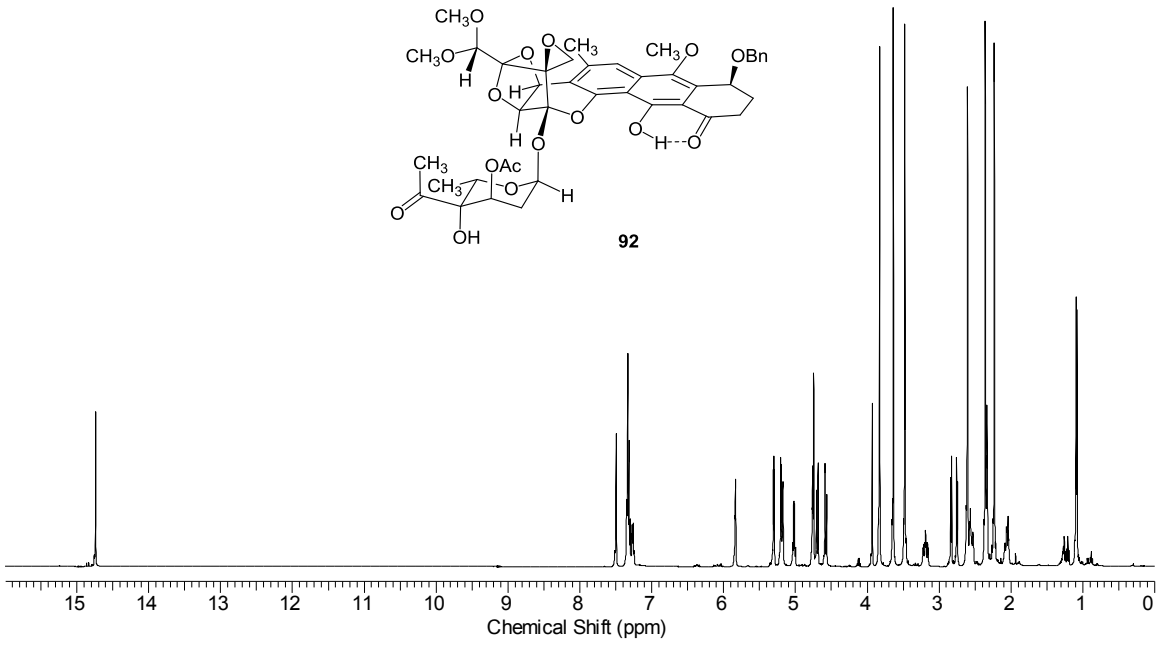

15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)


200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

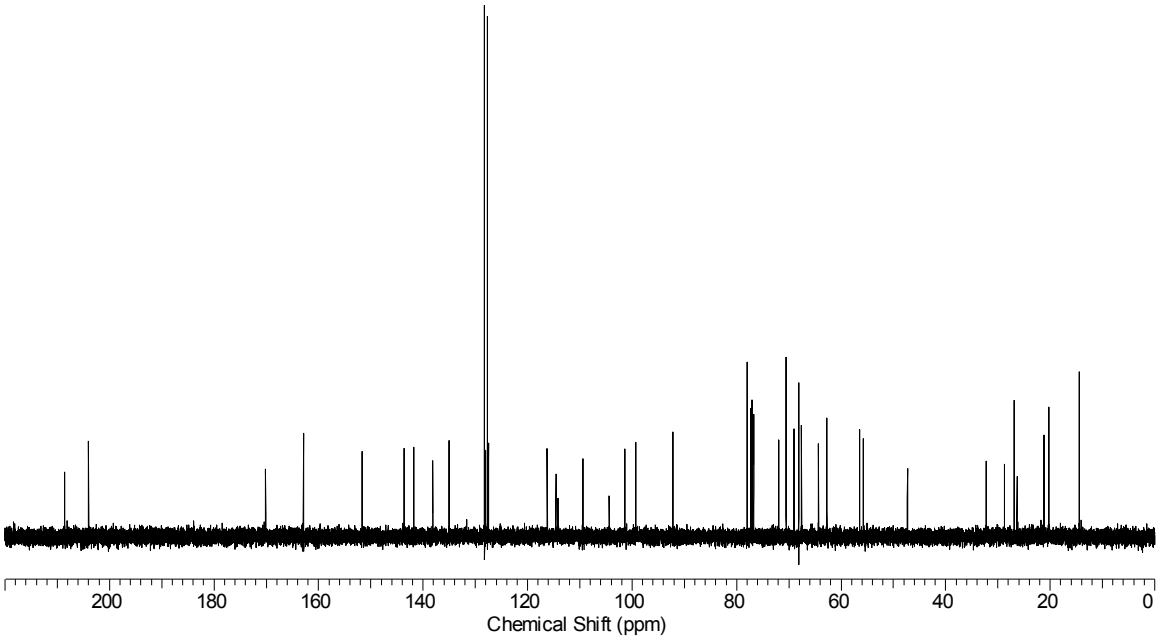

15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0
Chemical Shift (ppm)

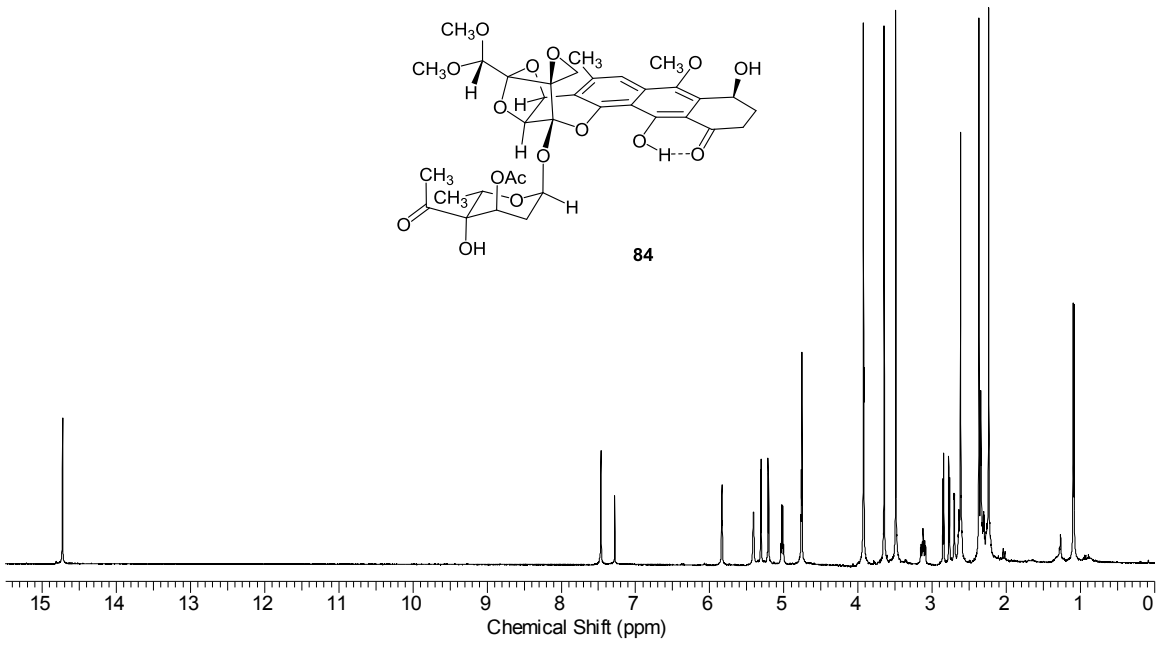
**90**


200 180 160 140 120 100 80 60 40 20 0
Chemical Shift (ppm)

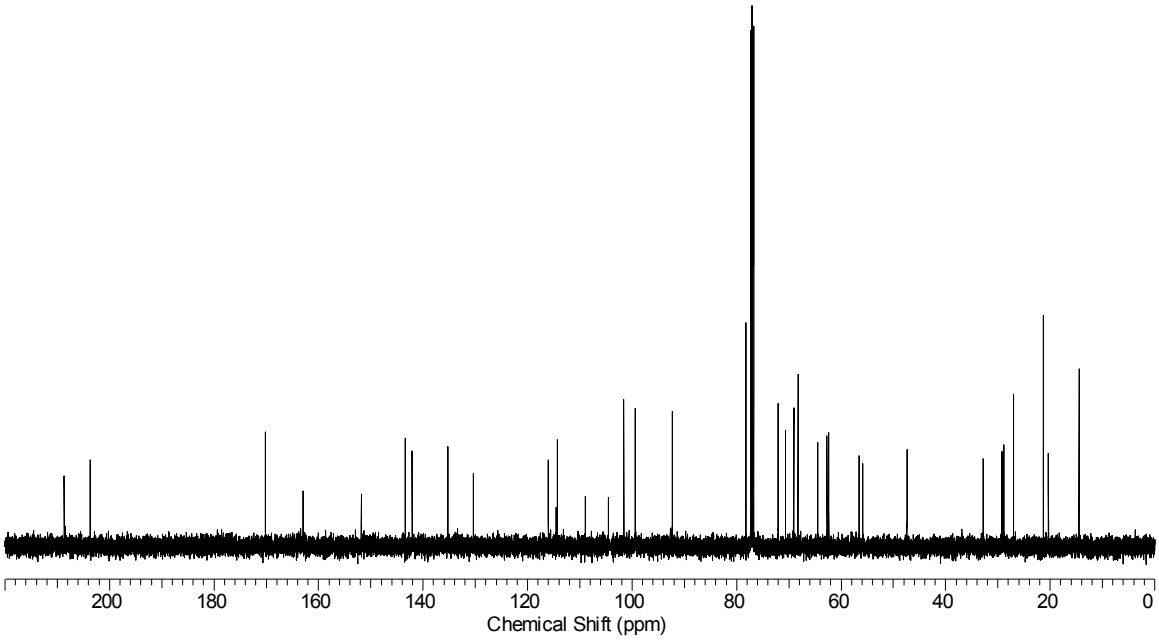

CH3O
CH3O
H
O
O
CH3
CH3O
OBn
O
H
O
H
O
O
H
O
H
O
CH3
OAc
CH3
O
H
O
OH
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

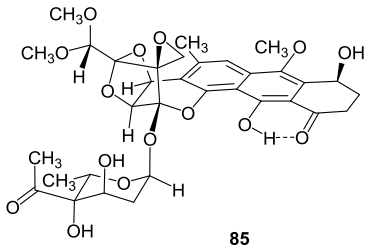
92


200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)


15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0
Chemical Shift (ppm)

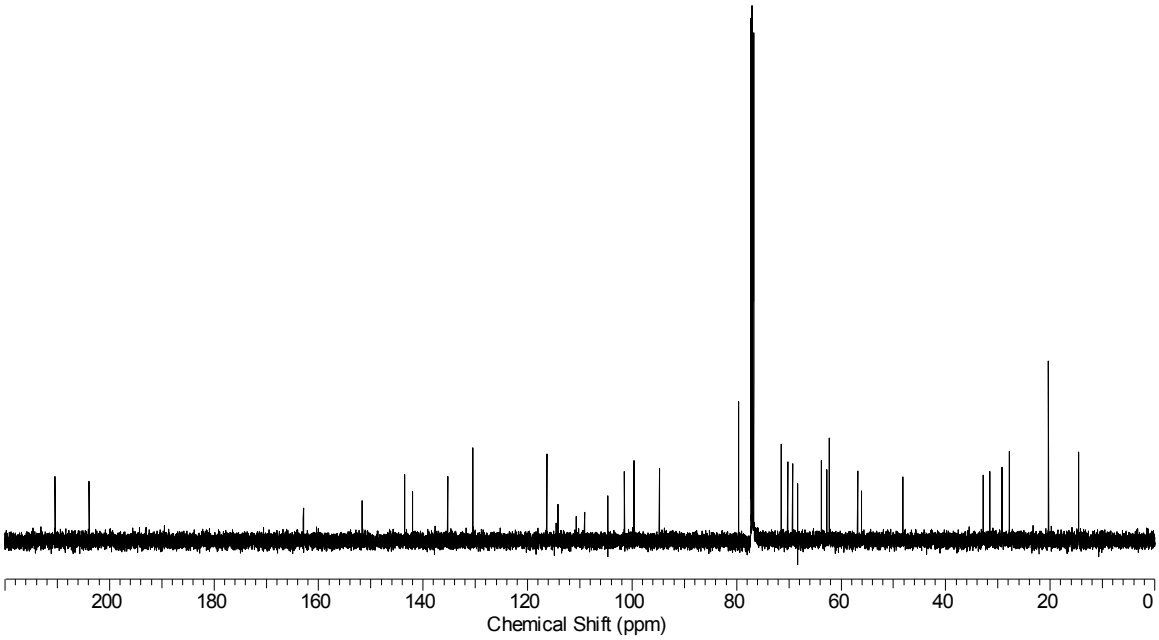
84

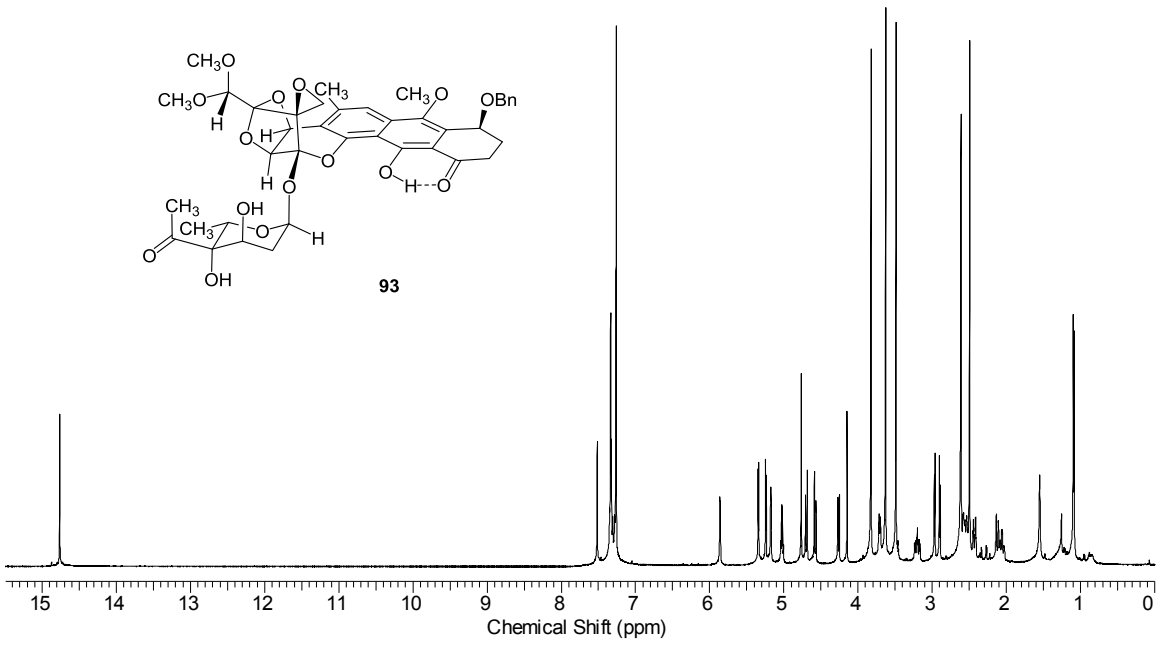

200 180 160 140 120 100 80 60 40 20 0
Chemical Shift (ppm)



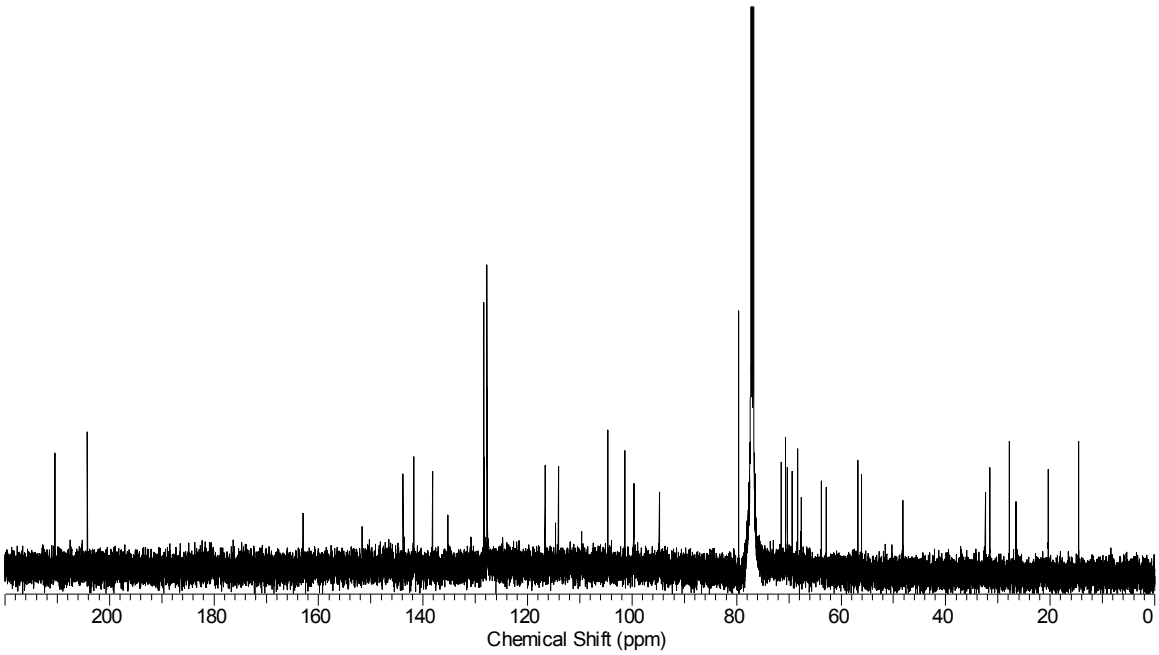
**85**

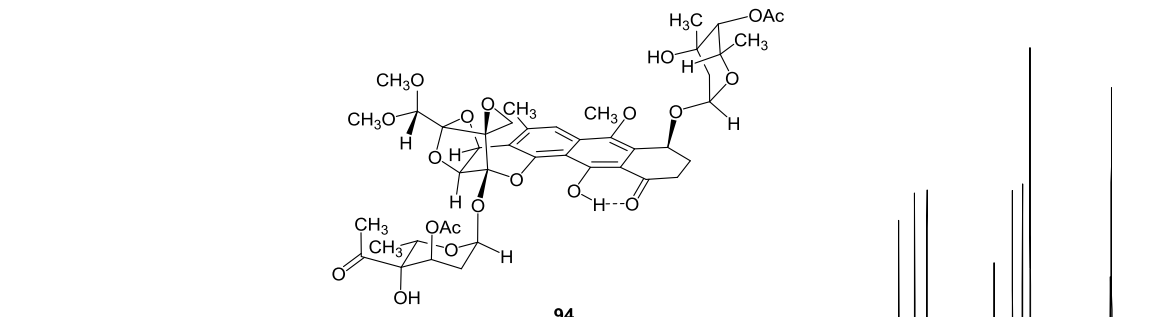

15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)


200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

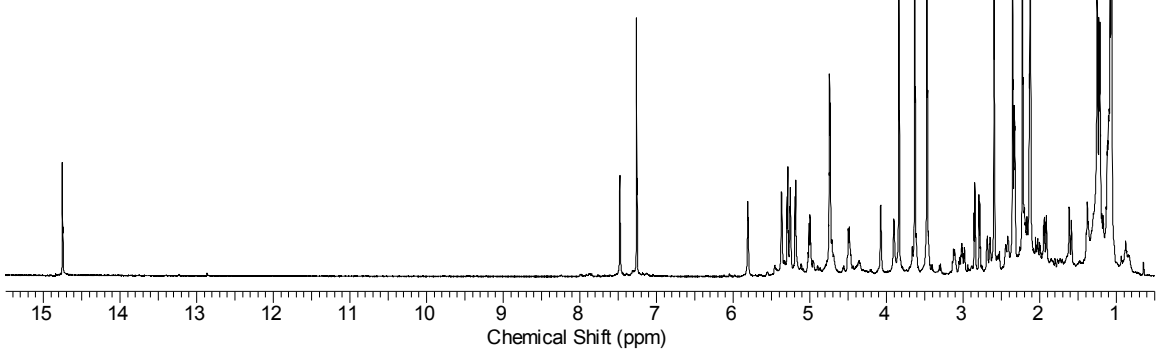

15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

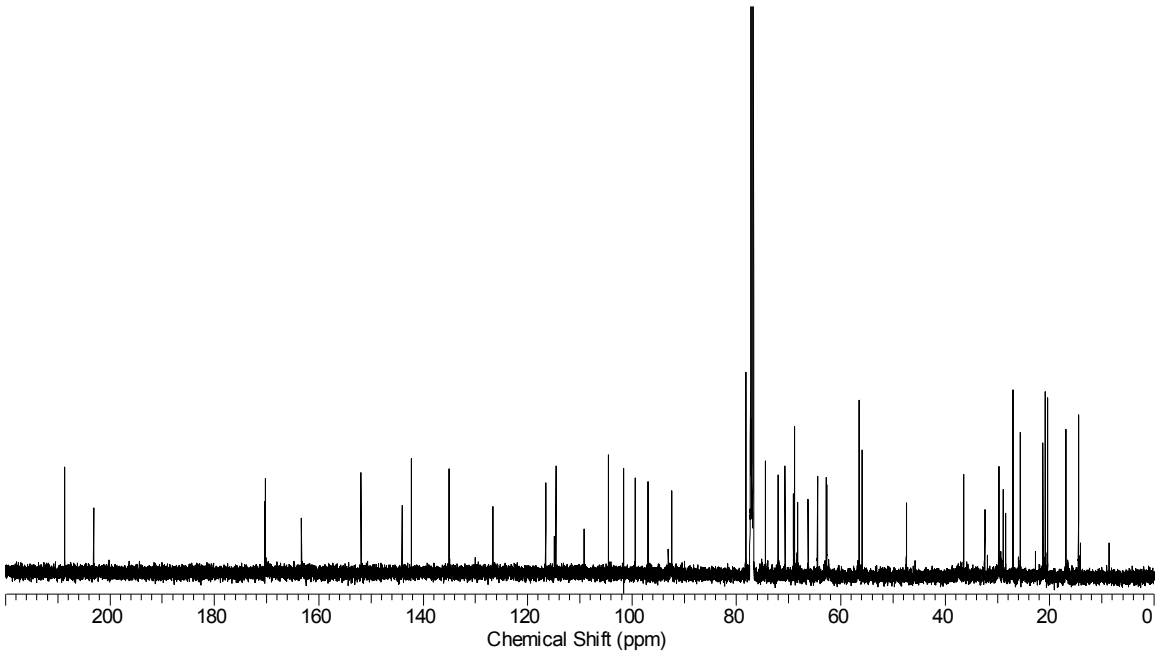
**93**

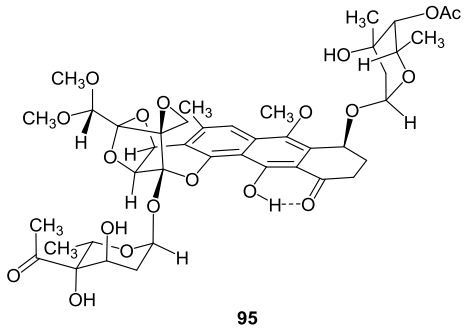

200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)



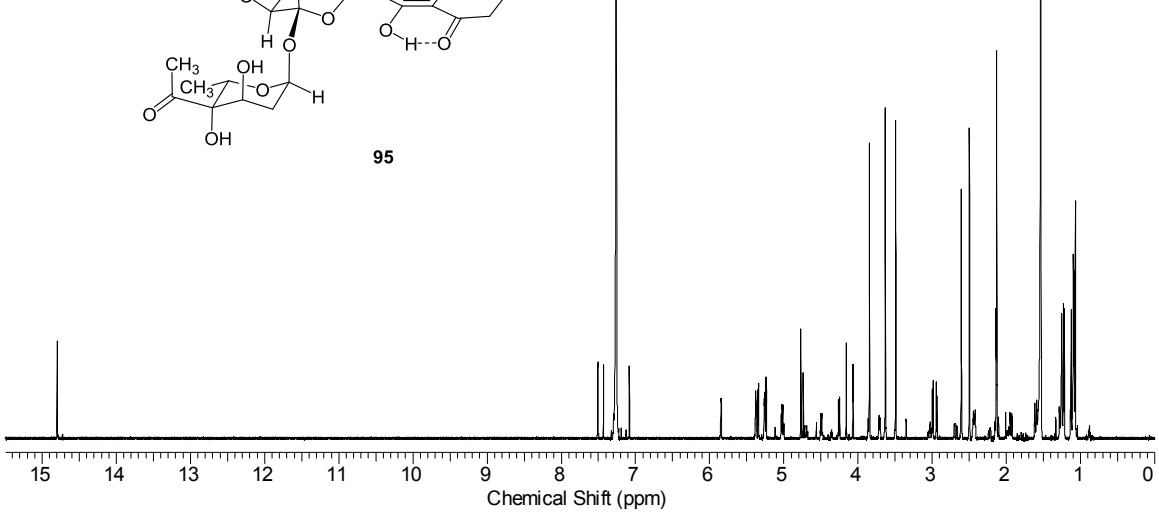
**94**

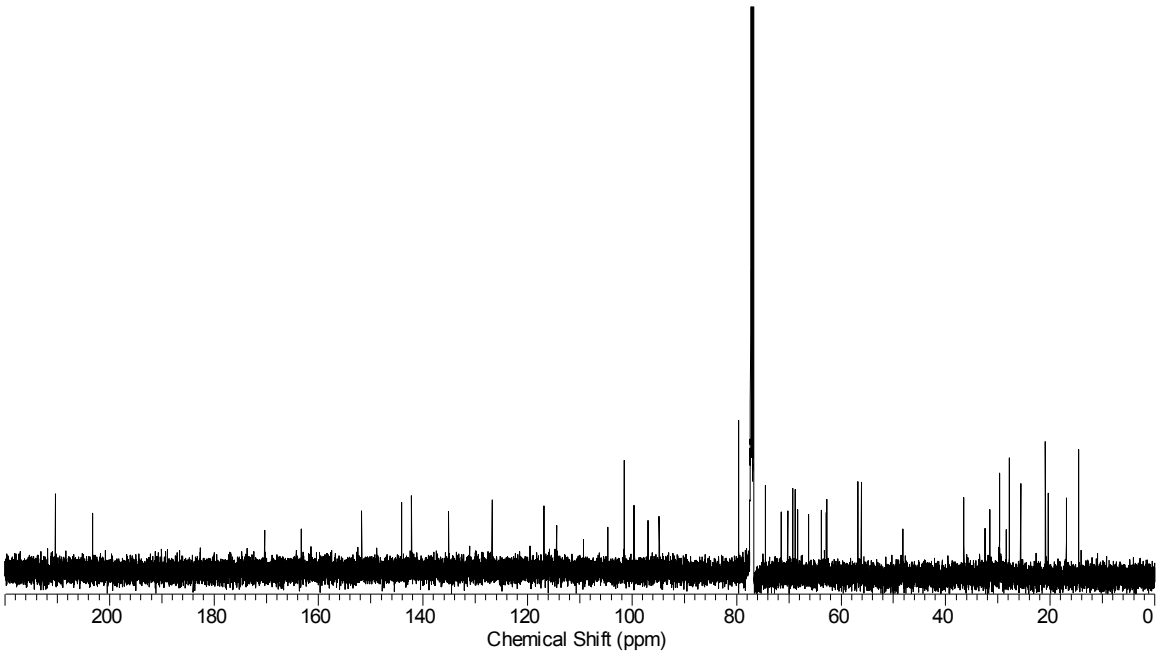

15 14 13 12 11 10 9 8 7 6 5 4 3 2 1
Chemical Shift (ppm)

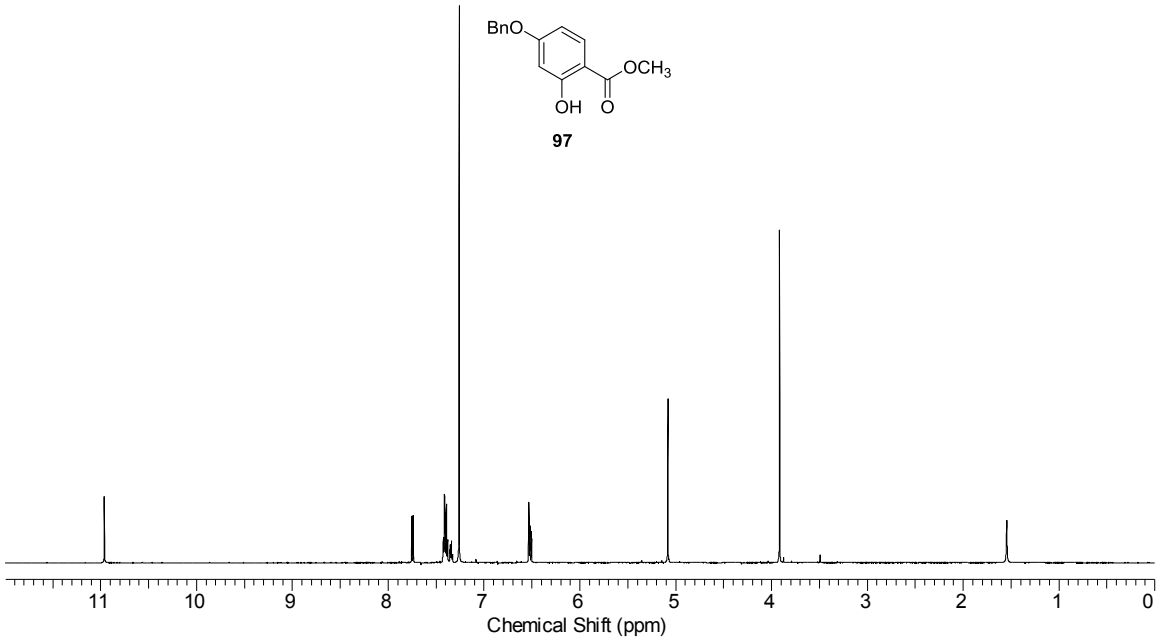

200 180 160 140 120 100 80 60 40 20 0
Chemical Shift (ppm)

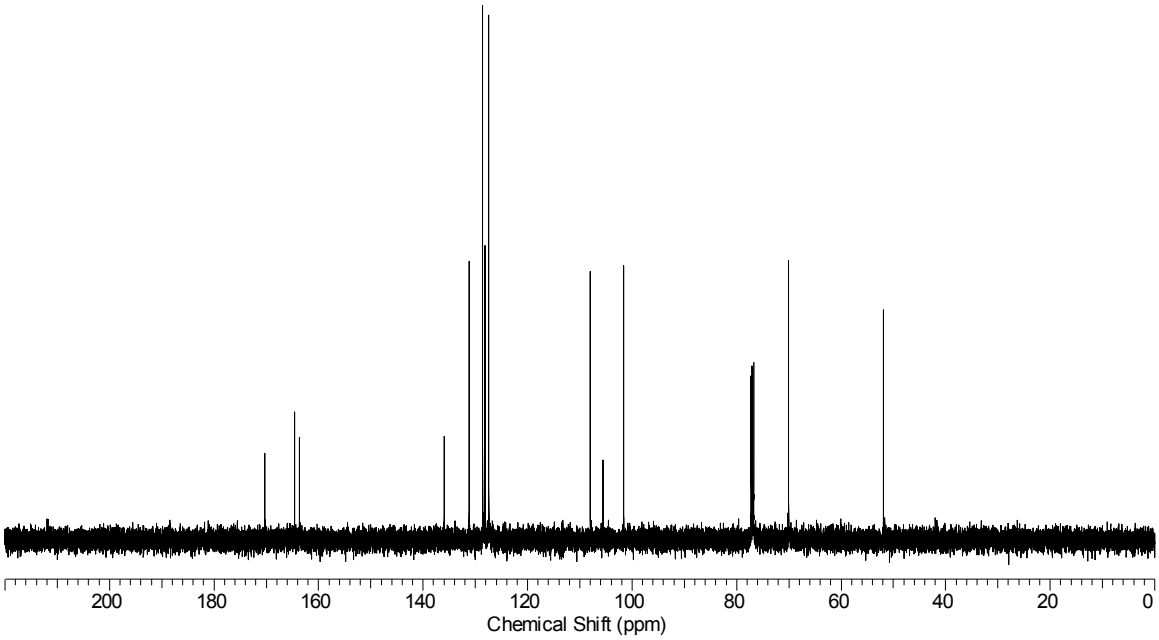


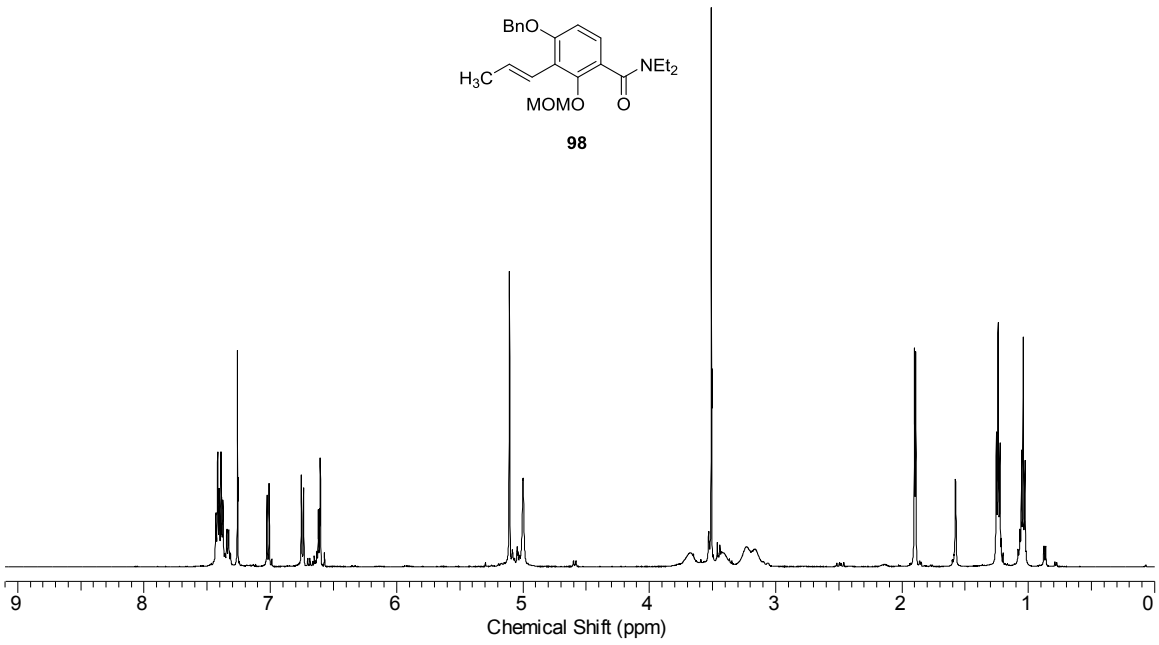
**95**

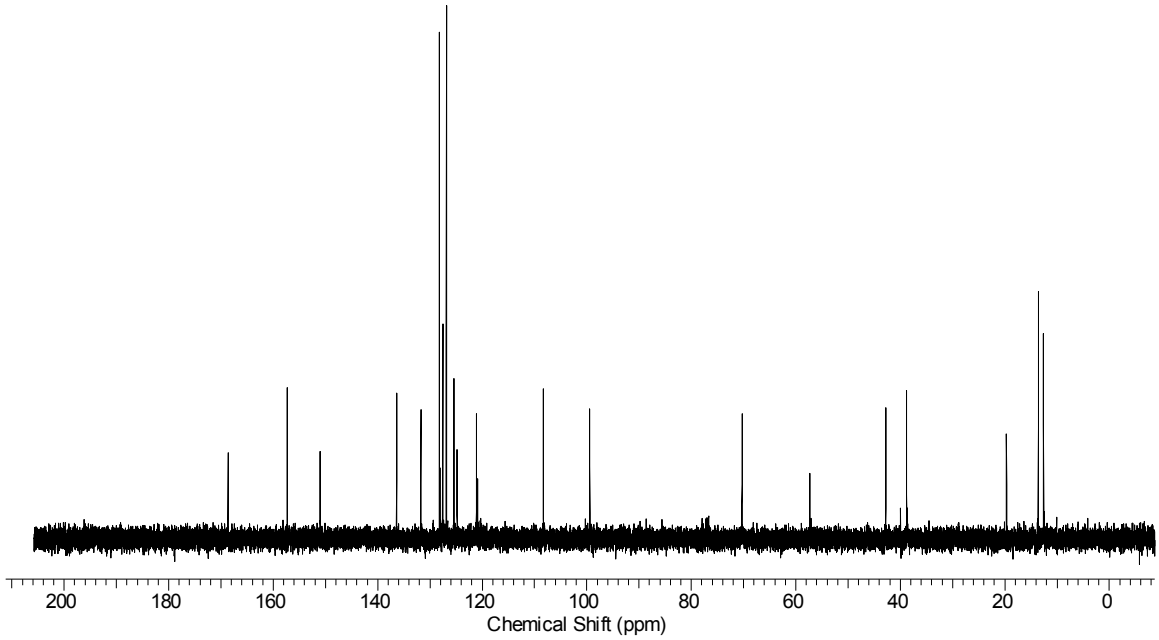

15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0
Chemical Shift (ppm)

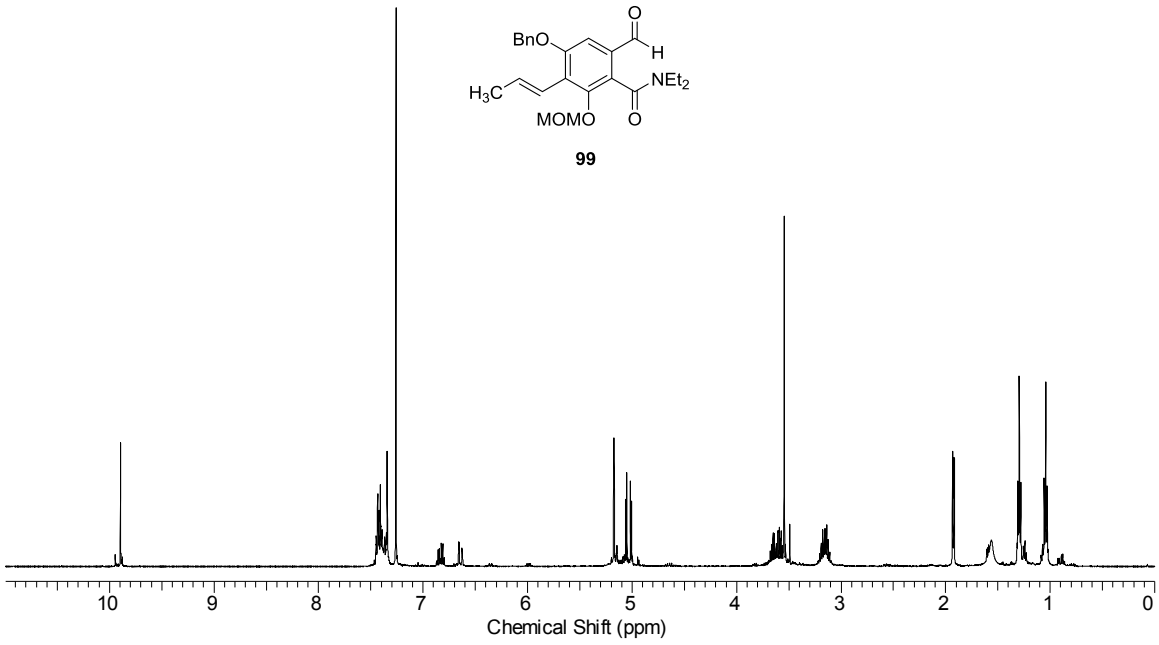

200 180 160 140 120 100 80 60 40 20 0
Chemical Shift (ppm)

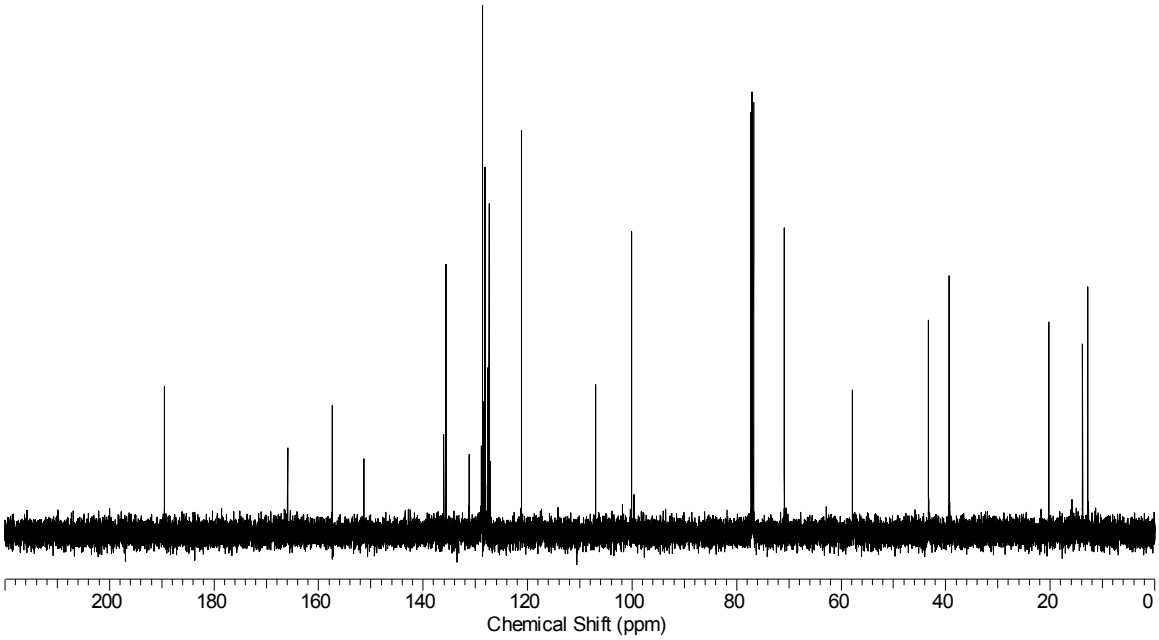

BnO
OCH3
OH
O
97
11
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

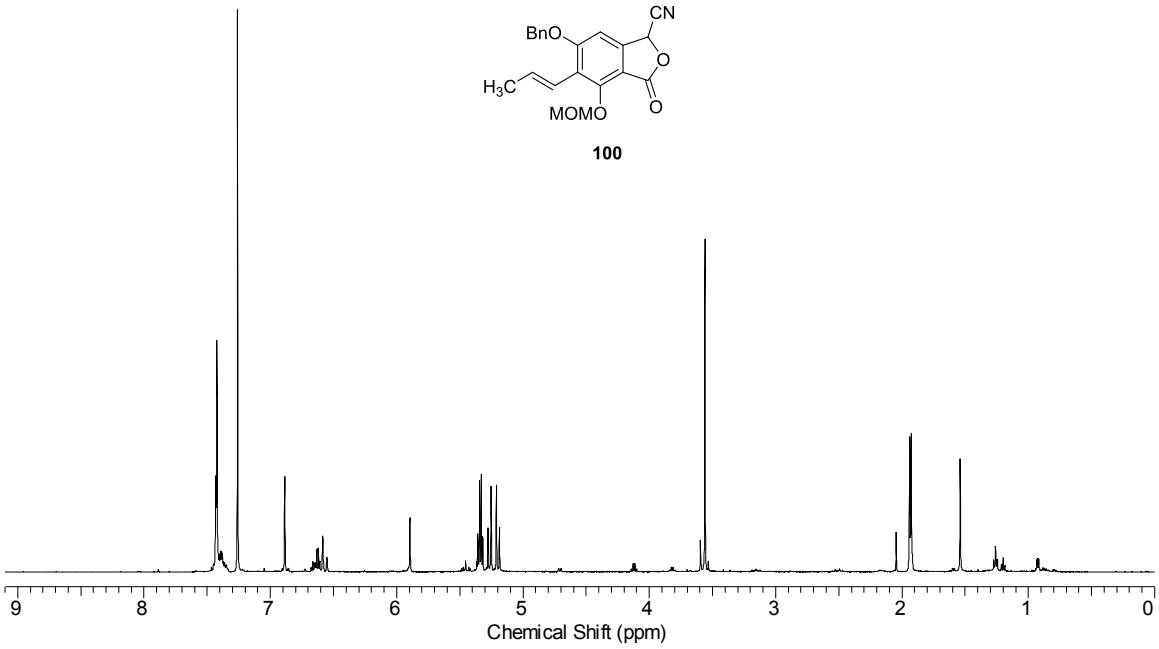

200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

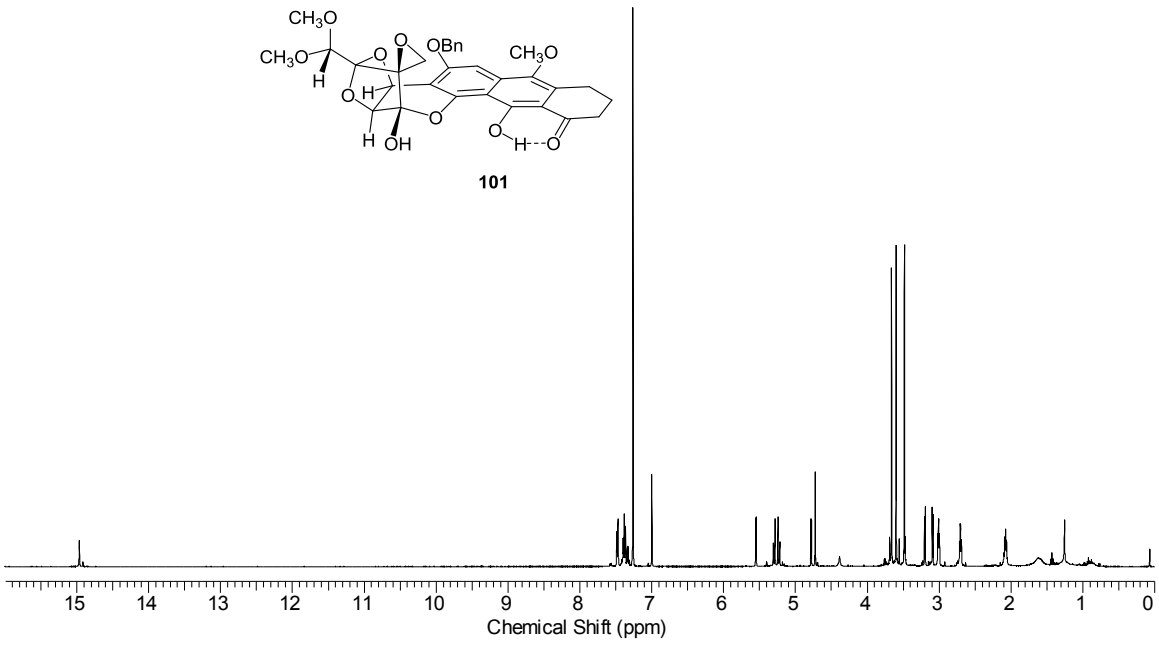

BnO
NEt2
H3C
MOMO
O
98
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)


200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

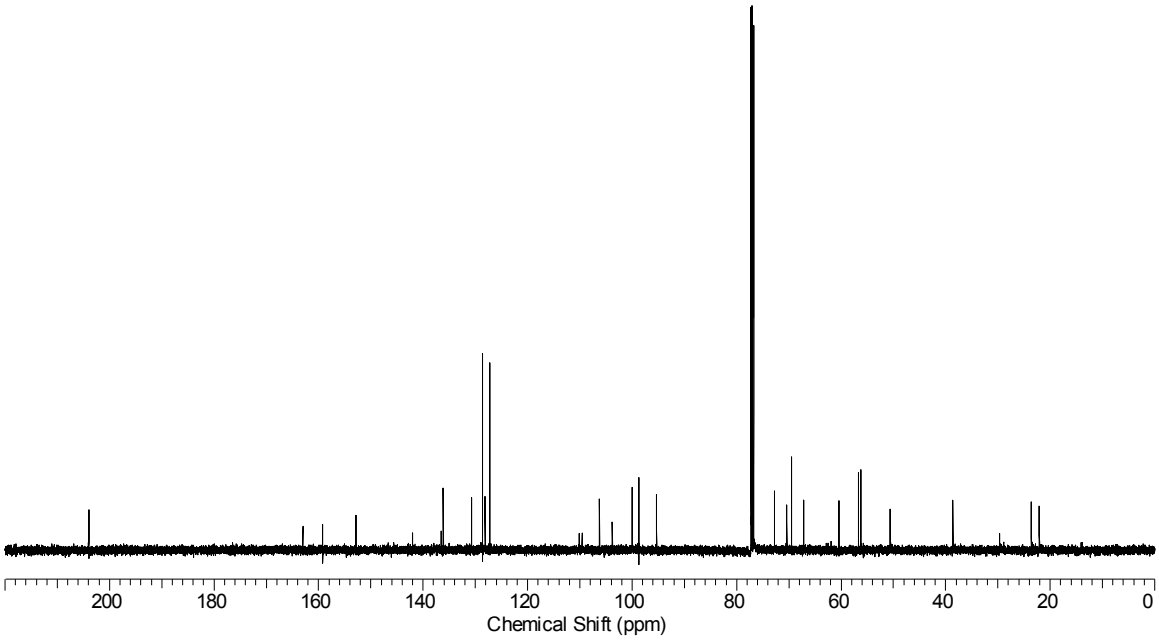

BnO
O
H
NEt2
H3C
MOMO
O
99
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

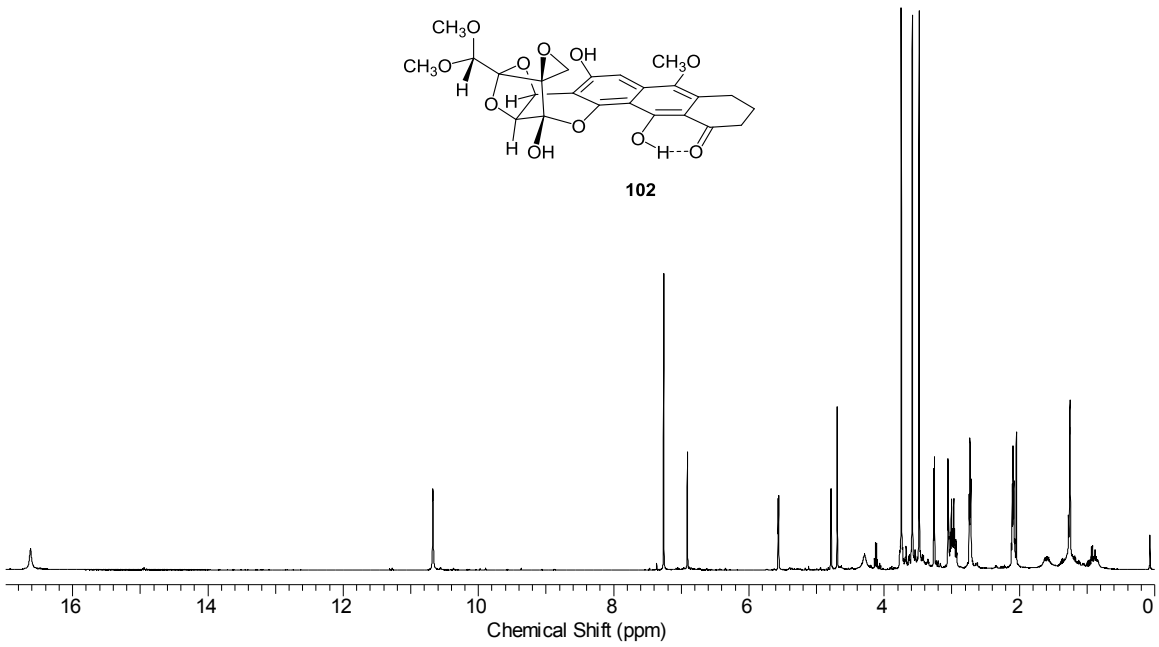

200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

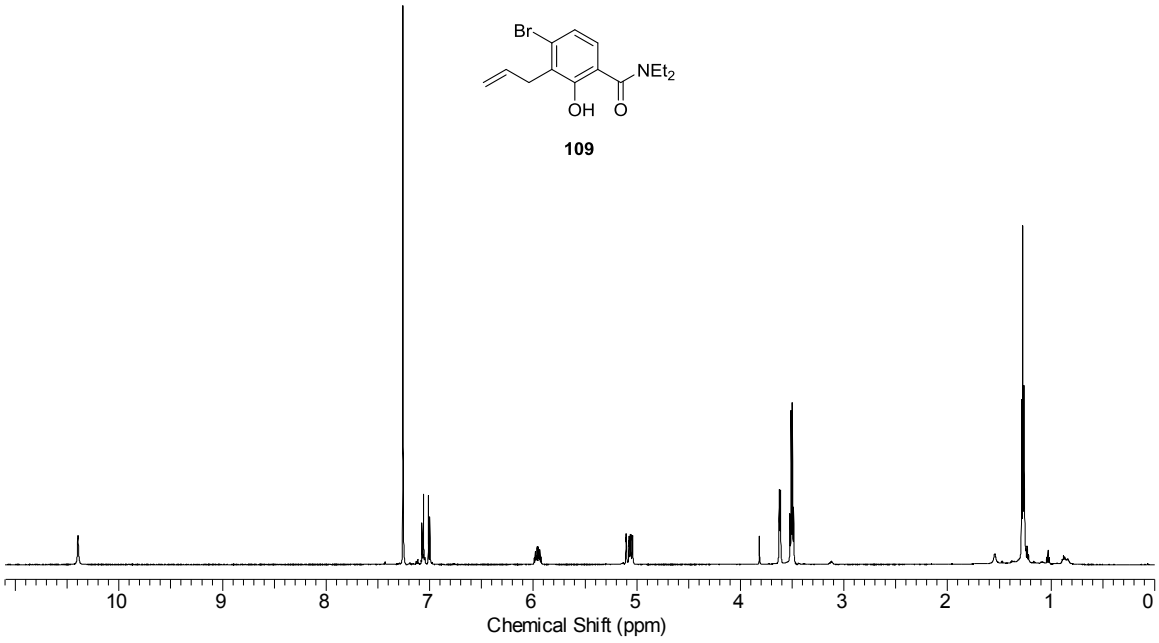

CN
BnO
O
H3C
O
MOMO
100
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

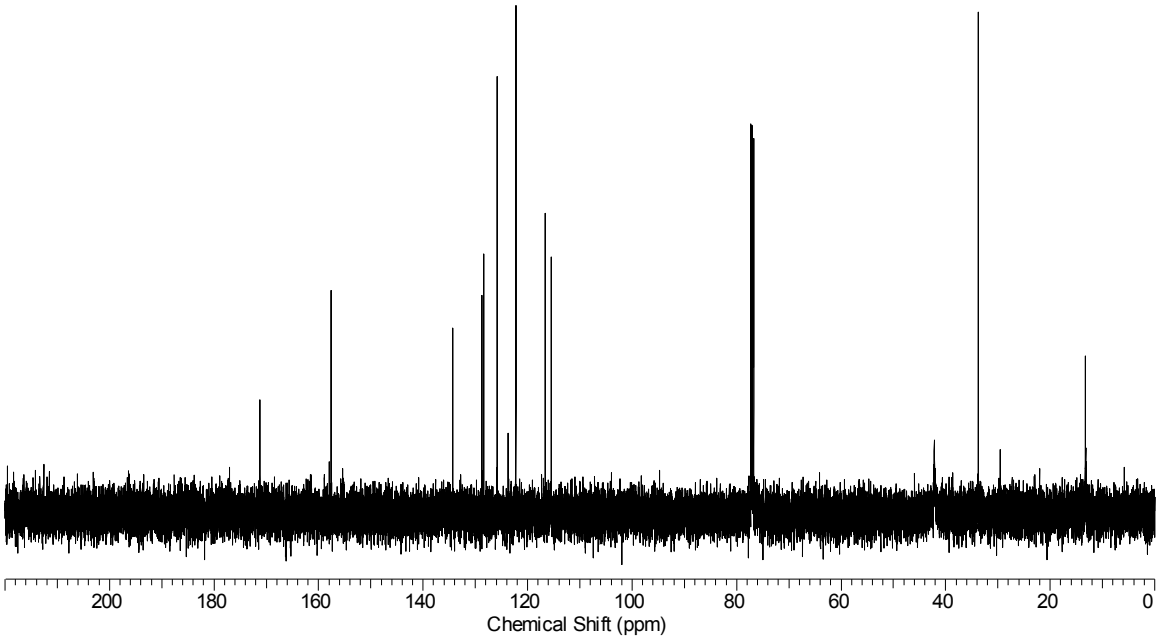

CH3O
CH3O
H
O
O
OBn
CH3O
O
H
H
O
H
OH
O
O
H---O
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

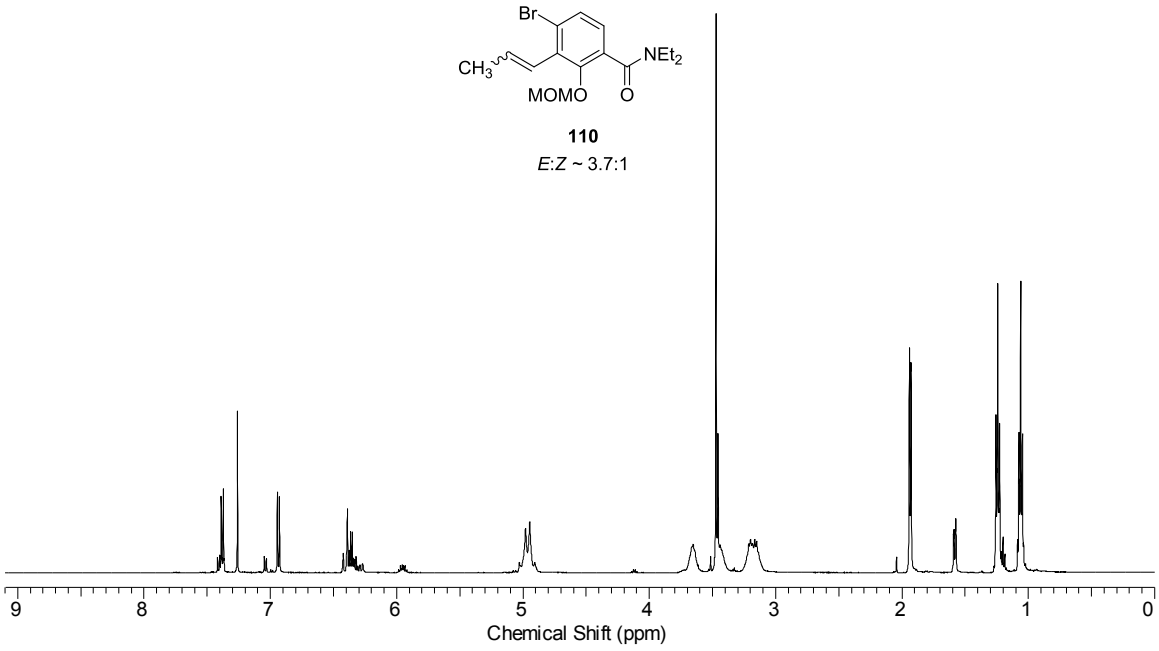
101

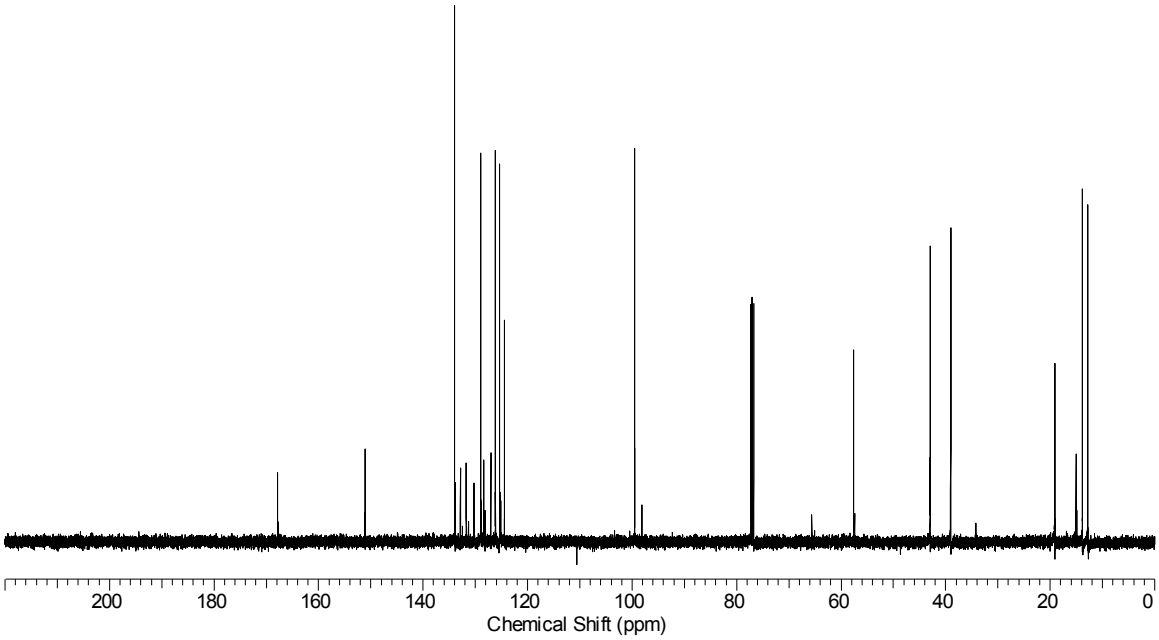

200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

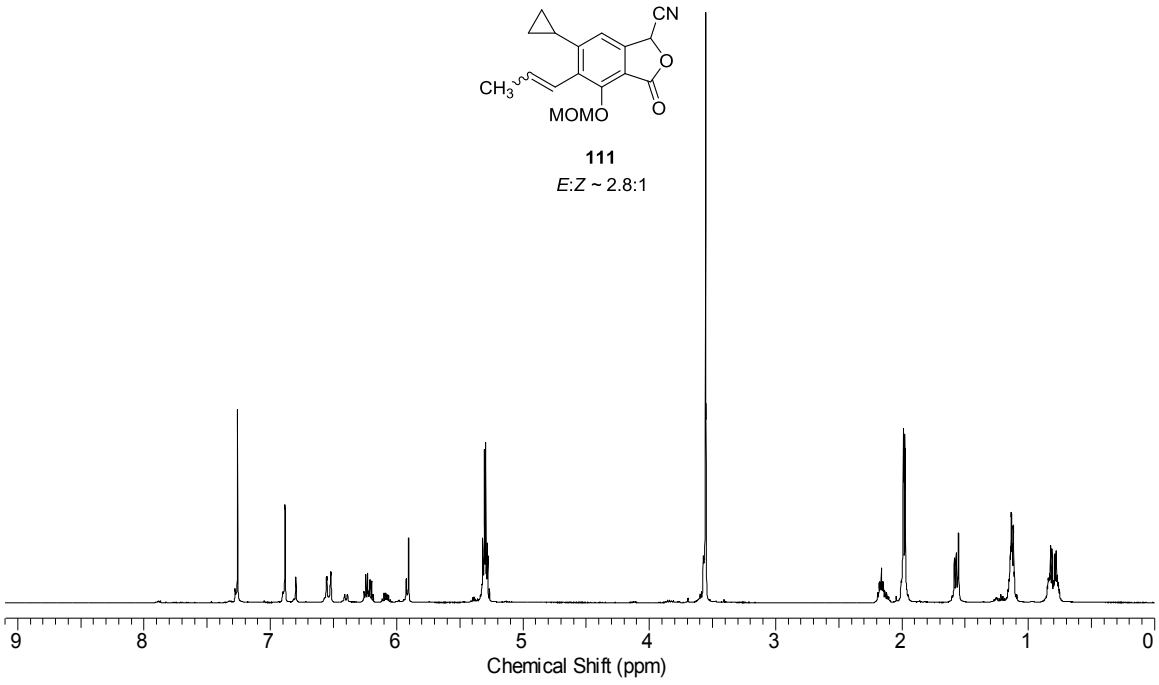

CH3O
CH3O
H
O
OH
CH3O
O
H
O
H
H
OH
O
H
O
102
16
14
12
10
8
6
4
2
0
Chemical Shift (ppm)

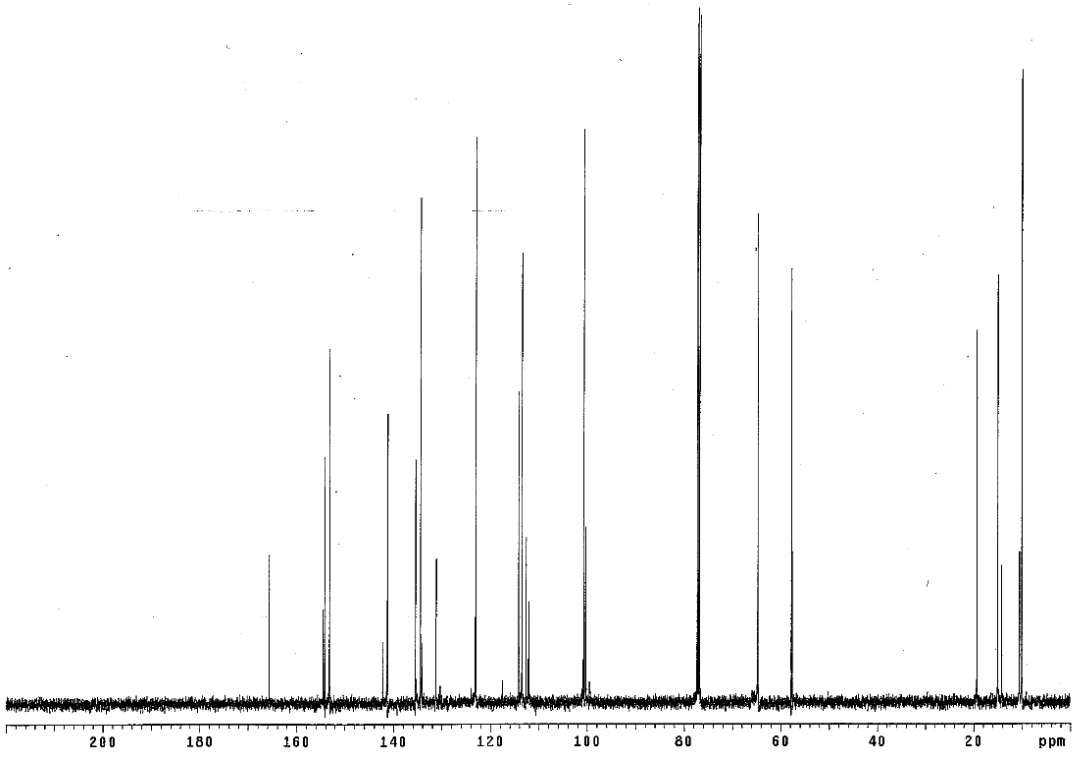

Br
NEt2
OH
O
109
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

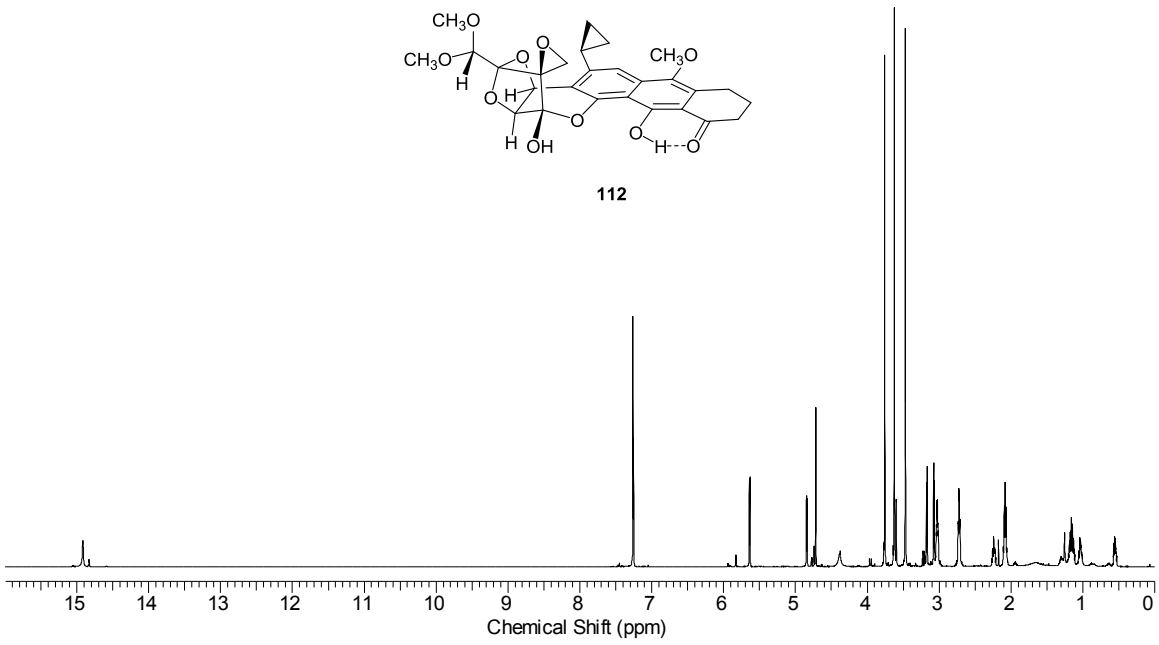

200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)


Br
NEt2
CH3
MOMO
O
110
E:Z ~ 3.7:1
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

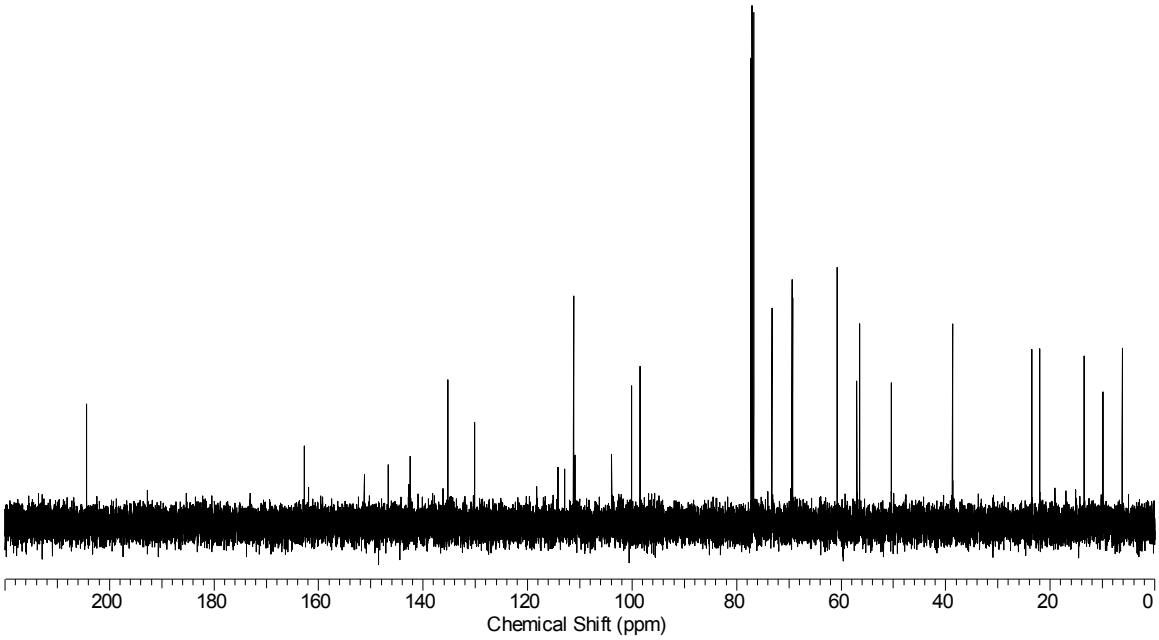

200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

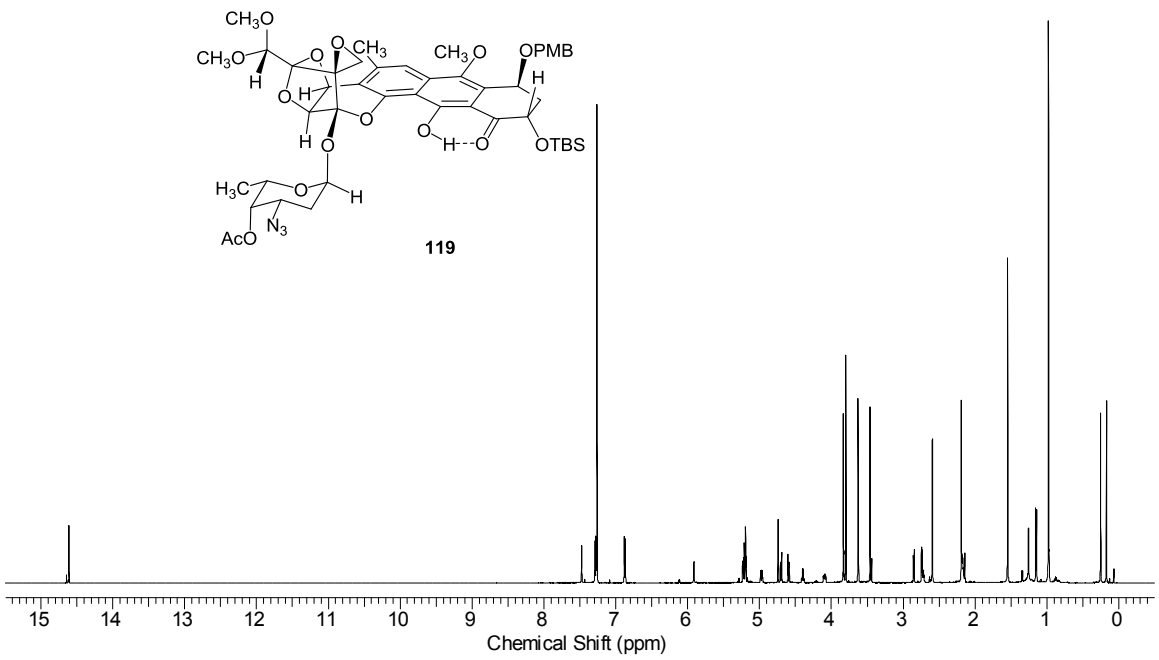

CN
O
O
CH3
MOMO
111
E:Z ~ 2.8:1
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)


200
180
160
140
120
100
80
60
40
20
ppm

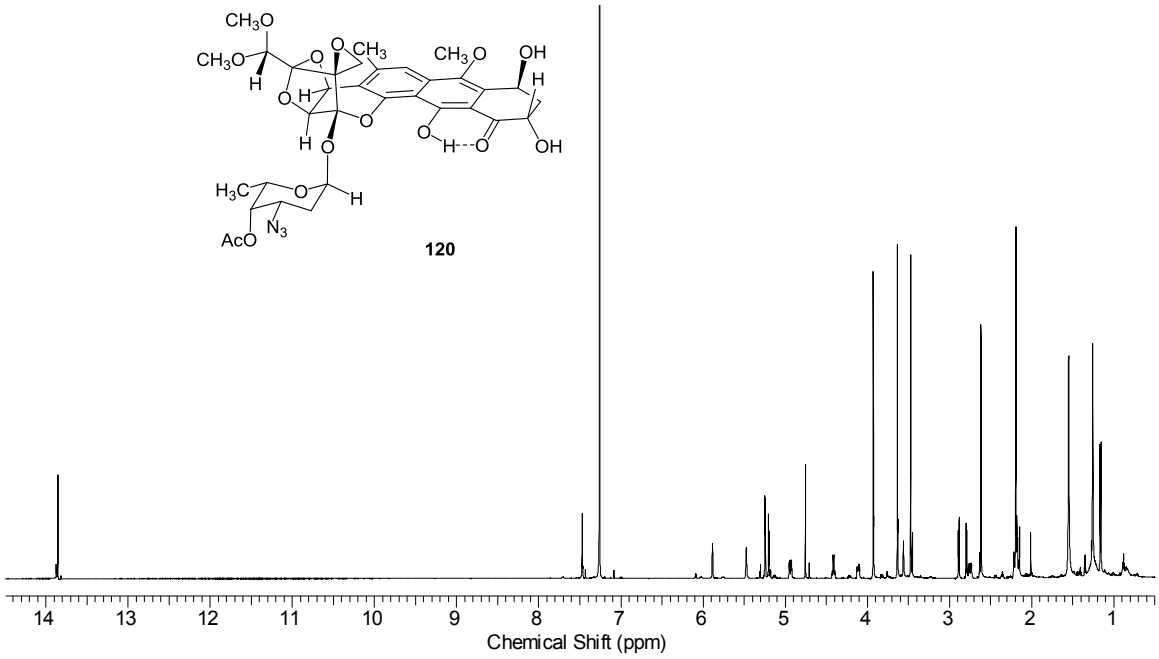

CH3O
CH3O
H
O
O
O
H
H
OH
O
CH3O
O
H
O
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

**112**


200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

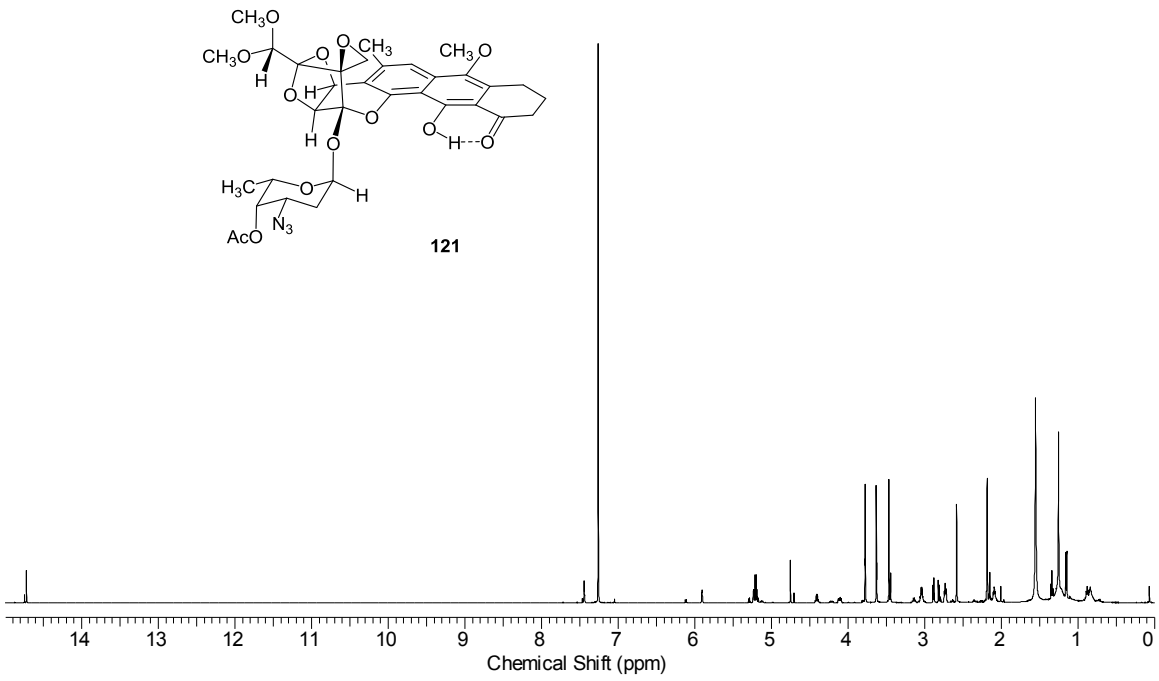

CH3O
CH3O
O
CH3
CH3O
OPMB
H
O
H
O
O
H
O
H
O
H---O
OTBS
H3C
O
H
N3
AcO

**119**

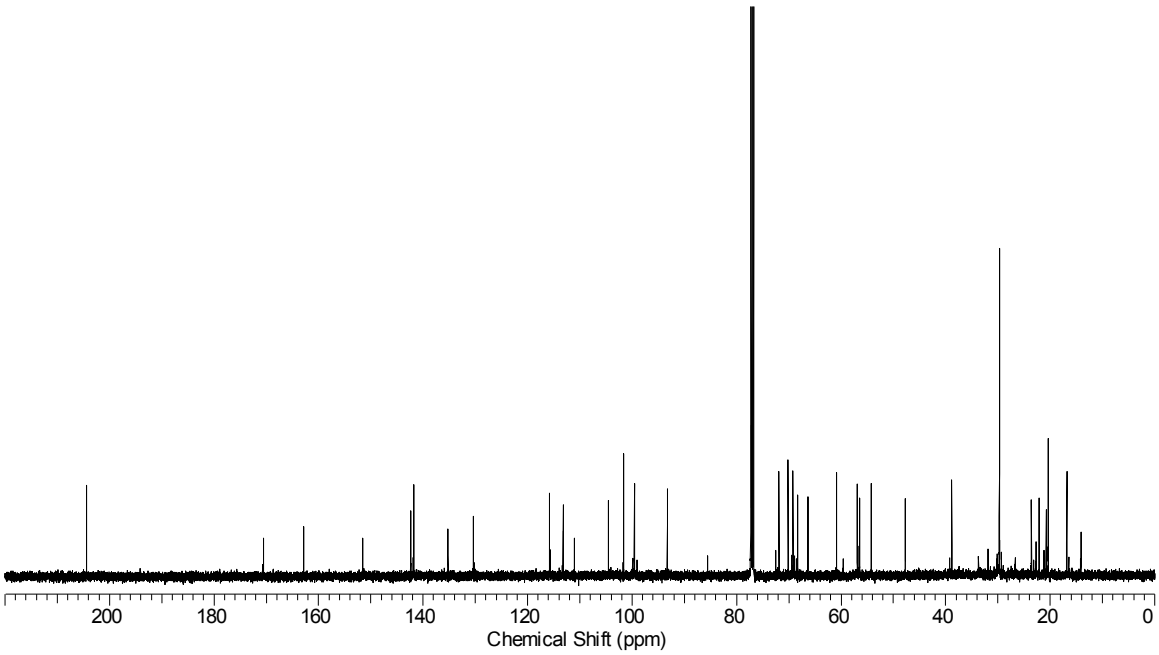

15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

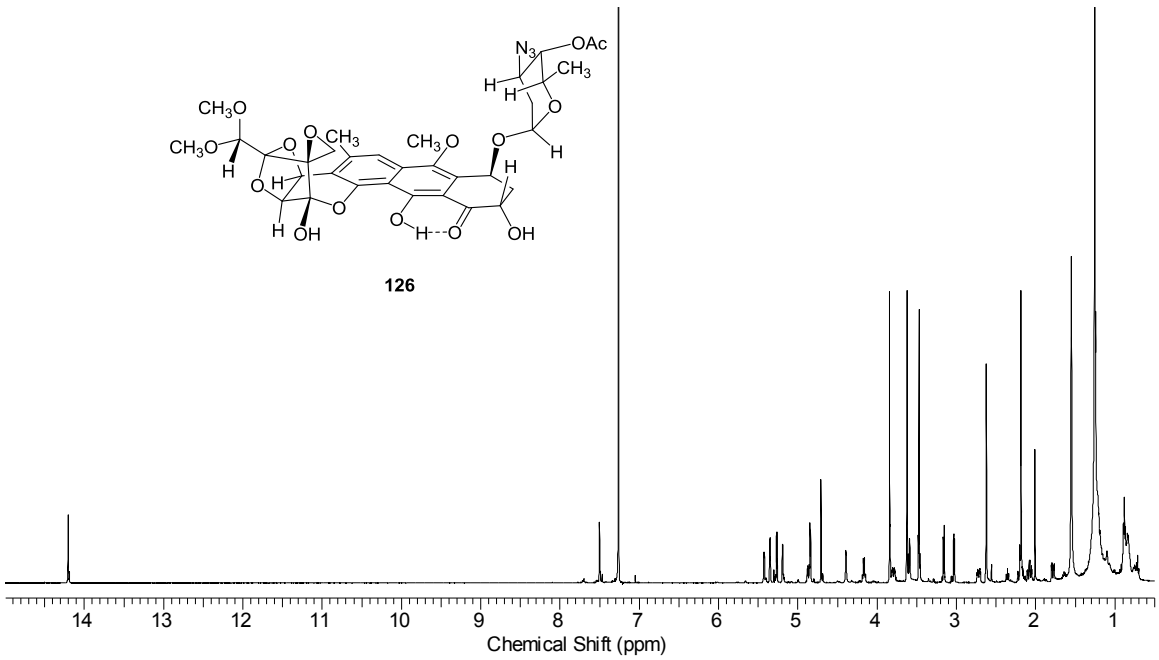

CH3O
CH3O
O
CH3
CH3O
OH
H
O
H
O
O
H
O
H---O
OH
H3C
O
H
N3
AcO
14
13
12
11
10
9
8
7
6
5
4
3
2
1
Chemical Shift (ppm)

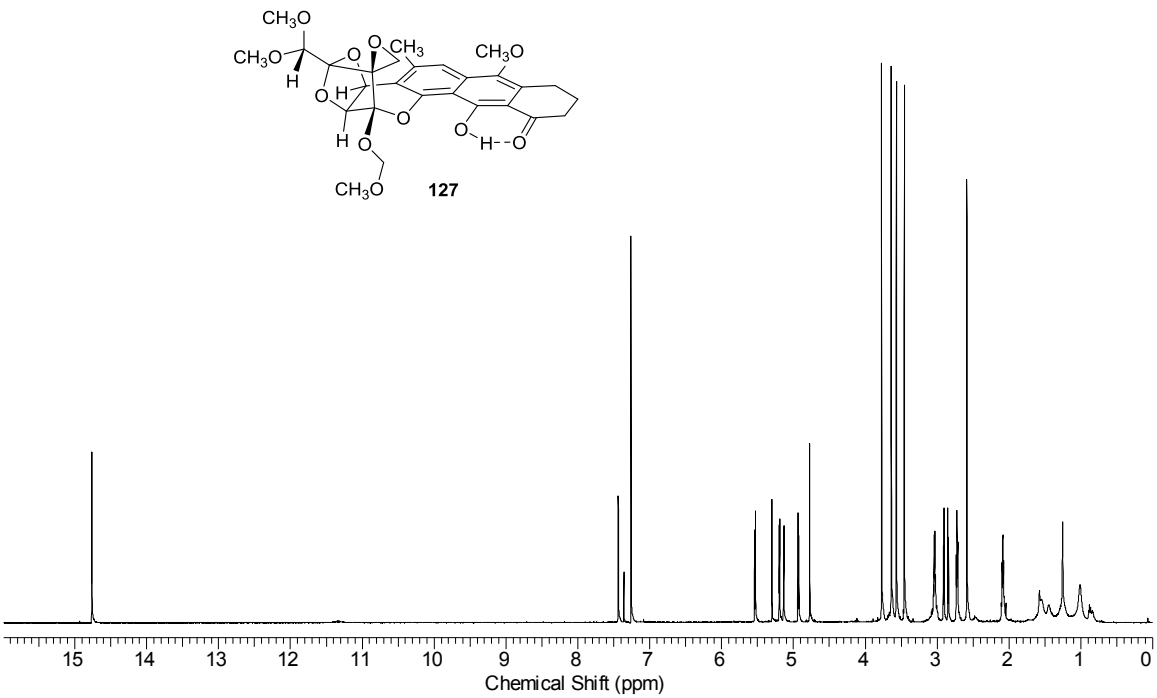
**120**

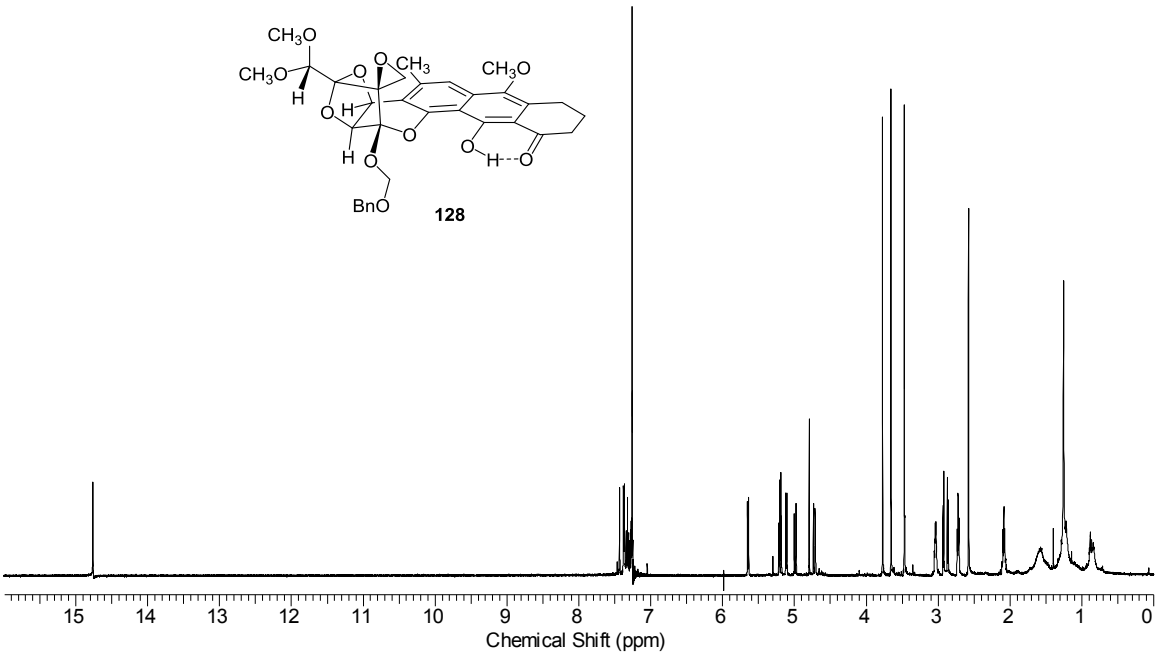


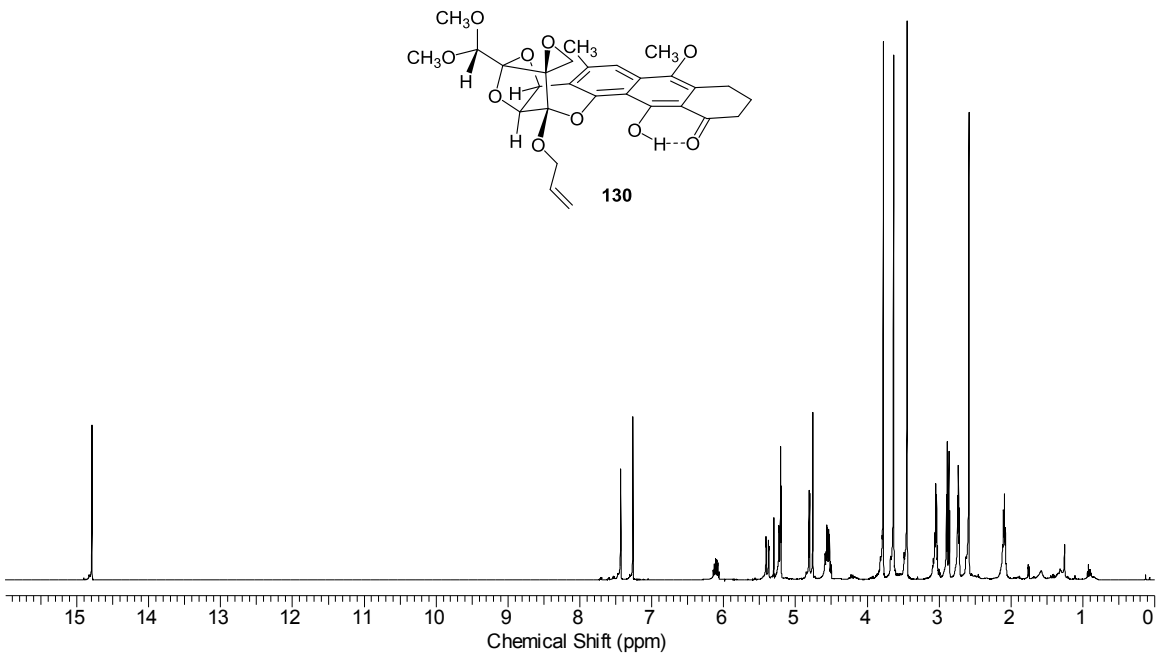

**121**



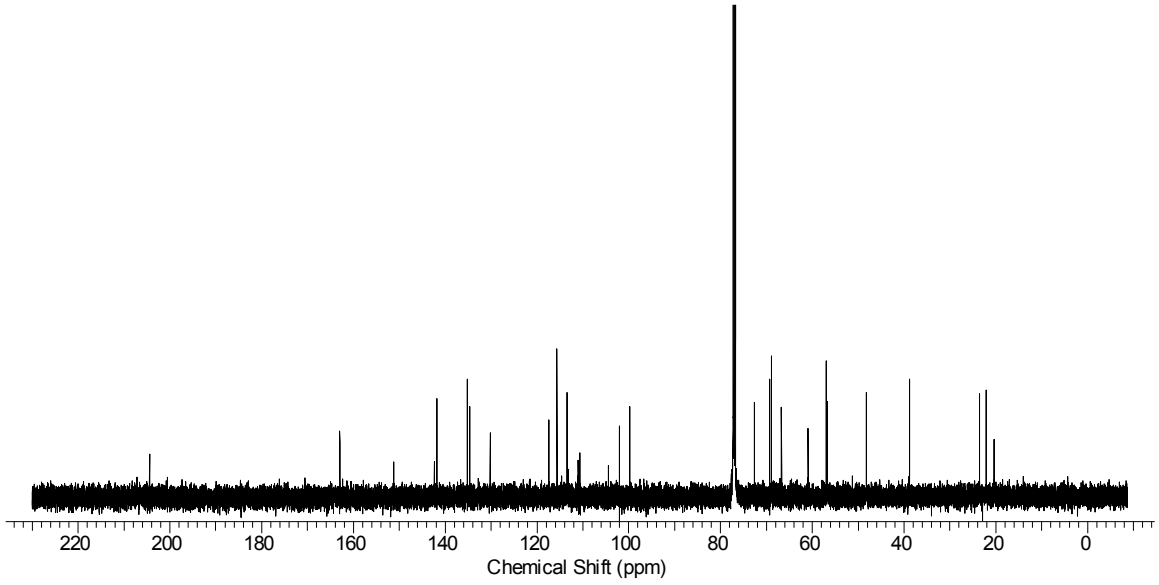

N3
OAc
CH3
H
H
O
CH3O
CH3O
O
O
CH3
CH3O
O
H
H
O
H
O
H
OH
O
H···O
OH
126
14
13
12
11
10
9
8
7
6
5
4
3
2
1
Chemical Shift (ppm)

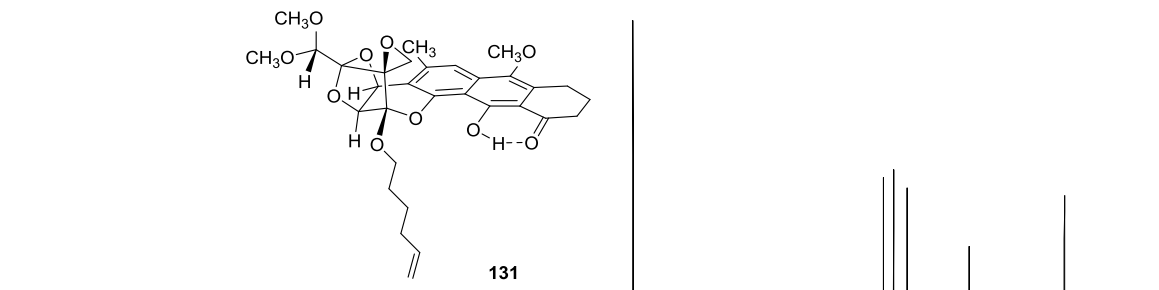

CH3O
CH3O
H
O
O
CH3
CH3O
O
H
H
O
O
H
O
O
H--O
CH3O
127
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)


CH3O
CH3O
H
O
O
CH3
CH3O
O
H
H
O
O
H
O
O
H--O
BnO
128
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)


15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0
Chemical Shift (ppm)

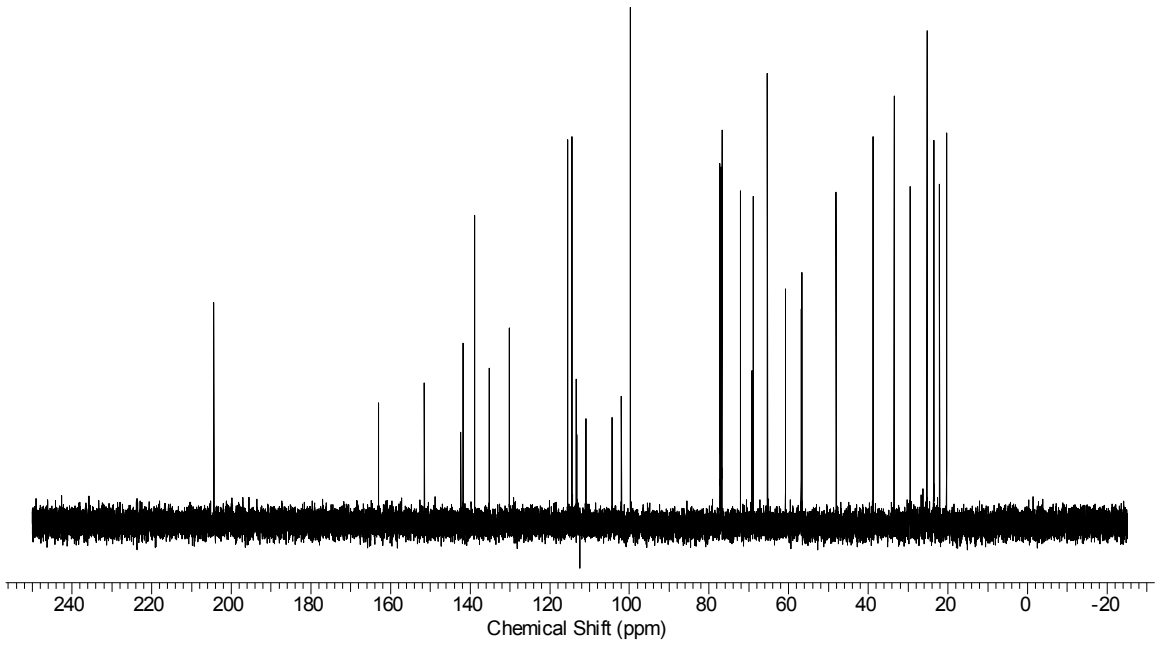
130


220 200 180 160 140 120 100 80 60 40 20 0
Chemical Shift (ppm)



131


15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0
Chemical Shift (ppm)


240 220 200 180 160 140 120 100 80 60 40 20 0 -20
Chemical Shift (ppm)